

VIRTUAL MICROSCOPY FOR THE
ASSESSMENT OF COMPETENCY
AND TRAINING FOR MALARIA
DIAGNOSIS

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VIRTUAL MICROSCOPY FOR THE
ASSESSMENT OF COMPETENCY AND
TRAINING FOR MALARIA DIAGNOSIS

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DECLARATION

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or other qualification.

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DEDICATION

I would like to dedicate by thesis to
my family, for all their support
throughout my studies.

GLOSSARY OF TERMS

ACT: Artemisinin based combination therapies
CPD: Continued professional development
DOP: Digital Outback Photo
EDTA: Ethylenediaminetetraacetic acid
FTP: File transfer protocol
GP: General practitioner
HTML: Hypertext Mark-up language
HRP-2: Histidine rich protein 2
IFA- Immunofluorescence antibody testing
Ig- Immunoglobulin
JPEG: Joint Photographic Experts Group- image format
LSTM: Liverpool School of Tropical Medicine
Mb: Megabyte
MPx: Megapixel
MRI: Manchester Royal Infirmary
NHS: National Health Service
NPV: Negative predictive value
PCR: Polymerase chain reaction
P.: *Plasmodium*
pLDH: Parasite lactate dehydrogenase
PPV: Positive predictive value
PS3: Photoshop CS3
QBC: Quantitative Buffy Coat
RAFT: Réseau Afrique Francophone de Télémédecine
RBC: Red blood cell
RDT: Rapid diagnostic test
ssrRNA: Small subunit ribosomal ribonucleic acid
SVS: ScanScope Virtual Slide- image format
SWF: Shockwave Flash format
TIFF: Tagged Image File Format – image format

UK NEQAS: United Kingdom National External Quality Assessment Scheme

UK NEQAS (H): United Kingdom National External Quality Assessment Scheme for General Haematology

USB: Universal Serial Bus

WBC: White blood cell

WHO: World Health Organization

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ABSTRACT

Microscopy is regarded by many healthcare professionals as the international gold standard for diagnosing malaria; however, the ability to reach a correct diagnosis is affected by training, experience and availability of laboratory resources including adequate quality assurance procedures.

In the work reported in this thesis we have generated virtual microscope slides from patients, with malaria for use as external quality assurance specimens. These virtual microscope slides were also incorporated into a training programme to improve the diagnosis of malaria in UK and International laboratories. In addition a novel gallery of photomicrographs taken from blood smears from various patients was used in the training programme.

Internationally, 40 participants were recruited from 14 laboratories recommended by the WHO, UKNEQAS (H) and the Liverpool School of Tropical Medicine. In the UK, a group of laboratory individuals was contacted through UK NEQAS (H) and 34 interested individuals were recruited.

Participants were initially asked to make a diagnosis on 40 electronically generated blood smear images to determine the presence, or absence, of malaria and to identify the species present. These participants were then given access to an Internet based training and quality assessment programme over a six-month period, aiming to improve malaria diagnosis by microscopy, before completing another assessment of 40 images.

In the initial assessment, 24 participants completed all 40 cases in the international and UK groups. In the final assessment 21 participants in the international group completed all 40 cases and 18 participants in the UK group. For the comparison of the initial and final assessments the results of 18 and 13 participants from the international and UK groups respectively were analysed.

In the initial assessment, the international group achieved the correct diagnosis in 76.4% of cases, and the correct species in 48.9%. The UK group achieved the correct diagnosis in 90.1% of cases and the correct species in 58.4%. In the final assessment the international group achieved the correct diagnosis in

72.7% and the correct species in 46.9% of cases. The UK group achieved the correct diagnosis in 95.6% of cases and the correct species in 73.8%.

The training programme resulted in a significant improvement ($p \leq 0.05$) in malarial diagnosis in the UK group, but the difference was not significant for the International group. The reasons for not being effective in Developing Nations could be due to difficulties in understanding English, speed of Internet connection, computers being used or the compliance of the participants.

Chapter 1: Project introduction

1.1 Project aims

The diagnosis of haematological diseases such as malaria can be monitored using virtual microscopy. The work reported in this thesis describes a pilot study used to determine if the Internet and virtual microscopy can be used as a method of delivering training and quality assurance for malaria diagnosis. The project was funded by the World Health Organization (WHO) Department of Diagnostic and Laboratory Technology, and supported by the United Kingdom National External Quality Assessment Scheme for General Haematology (UKNEQAS (H)), Manchester Royal Infirmary (MRI) and Liverpool School of Tropical Medicine (LSTM).

The overall aim of the project was:

To improve the diagnosis of malaria in the UK and Internationally using the Internet as a training tool, and as a provider of EQA to assess and improve competence

There were a number of objectives:

- To provide high quality digital images for use in quality assessment to take the place of EQA material
- To assess malaria microscopy in the UK and Internationally using the internet as a provider of a virtual microscope
- To determine to what extent sample variables such as artefacts and film preparation affect the diagnosis
- To analyse malaria diagnosis at different hospitals within the UK and Internationally to determine if there are any differences
- To assess internet access at the different participating sites, and determine if virtual microscopy is suitable for use in maintaining and improving standards of accuracy in malarial diagnosis.

To achieve these objectives the intervention study was designed to have three stages.

1. The initial assessment, this assessed the baseline competency in malaria diagnosis, and acted as the initial starting point on which further analysis was made.
2. The training stage, or the intervention, was provided between the two assessment stages. This was a combination of the virtual microscope and a web-based training programme.
3. The final assessment scheme was run in the same way as the initial, with these being compared to determine if the training had improved competency and the diagnosis of malaria.

1.2 Preparation and evaluation of material for digital microscopy

The initial assessment stage was designed to include images of blood smears that may be encountered in the day-to-day diagnosis of malaria. Sample variables were present, such as high numbers of platelets, staining artefact and other features that may lead to misdiagnosis. Both thick and thin films were used as these would be used in routine diagnosis. The exact method used was variable by location and laboratory, to account for this, each laboratory had the means to say that they normally did not use these slides. The images also represented all four malaria species that infect humans, along with negative samples and those with mixed infections. Forty high quality digital images were chosen for the initial assessment, to reflect the usual frequency of cases in the laboratory the majority of these were *Plasmodium falciparum*. These large stitched images, which are produced from 40 individual microscope fields, were used to assess the baseline competency. They were delivered over the Internet using the virtual microscope system designed by SlidePath Ltd. The high quality images provided a representative sample of EQA material.

Each case image was associated with a series of questions, these recorded the diagnosis made, comments on image quality and whether the slide would normally be used for diagnosis.

The answers provided by each participant were anonymous, each participant having an identification code, which was only known by a member of UKNEQAS (H) staff who was not directly involved in the project.

Following the initial assessment stage, the training programme was provided. This consisted of an interactive training package containing a gallery of images of individual parasite species and stages. These linked to larger images to simulate smear examination, in turn linking to stitched images, to represent the glass slide used in routine microscopic diagnosis. Along with these images, information about malaria in general and how each stage of the lifecycle is formed was provided, along with information detailing patient symptoms. Additional information pages covered good practice in sample preparation, in order to reduce pre-analytical variables. The training programme was provided in combination with annotated stitched images. The images previously viewed in the initial assessment were annotated showing where the parasites were present or in negative slides, artefacts that could have been confused with parasites. These annotated images were provided along with the answers the individuals provided when they answered the case.

Following the training programme, the final assessment stage was made available in the same way as the initial assessment. This provided a direct comparison of each participant's competency in diagnosis before and after the training programme to monitor if there had been any improvement. The participants were compared to their peer groups, in the same laboratory, as well as those in the same country and against all the participants involved. The images chosen for each assessment stage were comparable to prevent bias in the results from image selection.

1.3 Participant recruitment

Participants were recruited through UK NEQAS/ WHO, LSTM and via personal contacts.

The recruitment criteria used were

- Four different countries, aiming for one centre per country
- At least ten malaria diagnostic specimens examined per week
- Within laboratories all staff to participate, with one focal person acting as a trainer.
- This focal person must be willing to use the project material to teach other staff and/ or students.
- Participants would be required to dedicate less than three hours per month to analyses and must also dedicate time to training other staff.

On meeting the above criteria, the participants were then sent two types of questionnaire to complete. These questionnaires were, one for the laboratory manager to complete giving information about the procedures and methods followed in their laboratory. There was also a personal questionnaire which each participant completed asking about their training and experience. The minimum size for a significant result at $p \leq 0.05$ is 37.

Participants were recruited from four different African countries Ghana, Kenya, Malawi and Nigeria, in addition to laboratories, which were requested to participate by the WHO, in Chile, Colombia, Hong Kong, India, and Lebanon.

In total, forty participants from 14 laboratories were recruited onto the project. These laboratories were mainly at tertiary level hospitals, with some based within university research departments and others in private laboratories. Further details of the participants recruited are given in table 5.1.

UK participants were also recruited via UK NEQAS (H). Ten laboratories were chosen at random from a list of the participants receiving slides for the parasite quality assurance scheme. If the contact agreed, they were then emailed further

details and nominated members of staff who were interested. Thirty-four individuals were recruited onto the project. Further details of the participants recruited can be seen in table 5.2.

1.3.1 Participant Internet requirements

Internet access in the different locations was variable. Some laboratories had direct access to the Internet, while others had no computers. Internet access in Nigeria was particularly difficult and resource inputs were required to enable them to access the Internet. All other individuals had access to the Internet. The participants were also asked to ascertain that they could connect to the project site before committing to the project.

Chapter 2: Malaria diagnosis: Relevance to Practice in Endemic Regions

The accuracy of malaria diagnosis throughout the world is variable and somewhat unreliable (Amexo *et al.*, 2004). There are an ever-increasing number of methods available for diagnosis; this review highlights the methods available and their applicability for use in diagnosis, in countries where malaria is endemic.

2.1 Background

Malaria is one of the most common infectious diseases worldwide and the most important parasitic infection in humans (Greenwood *et al.*, 2005), causing an average of 189 – 327 million cases a year and 610,000 – 1,212,000 deaths annually (World Health Organization, 2008). The majority of deaths are in children and pregnant women (Williams, 2009). Malaria has a wide region of distribution, being found in most tropical areas, and is particularly prevalent in sub-Saharan Africa (Ashley *et al.*, 2006). Ninety per cent of malaria cases and deaths occur in sub-Saharan Africa, with young children and pregnant women being the most severely affected (Sherman, 1998).

Malaria is caused by a protozoan parasite of the genus *Plasmodium* (Ashley *et al.*, 2006). There are five different *Plasmodium* species that can infect humans, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. The vector for the transmission of malaria is the female *Anopheles* mosquito (Ashley *et al.*, 2006) when the mosquito takes a blood meal. In recent years cases of *P. knowlesi* a malaria species seen in monkeys, has been reported in humans (World Health Organization, 2010a). These cases have been mainly reported in South East Asia, with a number of deaths also reported (Cox-Singh *et al.*, 2008).

About 40% of the world's population is at risk from malaria infection, in some of the poorest countries (Amexo *et al.*, 2004), making treatment and diagnosis difficult due to a lack of adequate resources. Malaria in these regions is becoming increasingly difficult to treat due to the development of drug resistance, with innovative treatments being costly and with increased side-effects (Amexo *et al.*, 2004). As drug resistance has developed, treatments are

being altered; the new artemisinin drugs are becoming increasingly used. Artemisinin based combination therapies (ACTs) are now considered the best treatment by the WHO (World Health Organization, 2006).

2.1.1 Malaria species

Plasmodium falciparum

P. falciparum, also known as malignant tertian malaria, is associated with the most severe disease (Trampuz *et al.*, 2003). *P. falciparum* is the most highly pathogenic species, having an acute course of infection (Warrell and Gilles, 2002). Severe malaria classification is based on the clinical symptoms and causes the most deaths due to complications and organ involvement. Severe malaria is uncommonly seen with the other *Plasmodium* species, mainly due to the ability of *P. falciparum* to replicate in any age of cell at a rapid rate.

P. falciparum's lifecycle is the shortest of all the malaria species, rapidly leading to high parasite numbers and severe infection. Some species of malaria infect erythrocytes at a specific stage of development; *P. falciparum* however can infect all stages, leading to more erythrocytes being infected and more severe disease. Red blood cells that are infected with the parasite are also associated with clumping which can cause blockage of capillaries leading to organ damage (Warrell and Gilles, 2002). The major cause of death from malaria related conditions is cerebral malaria (Abdalla and Pasvol, 2004), caused by the aggregation of erythrocytes in the brain and blockage of capillaries.

P. falciparum is the predominate species in most endemic countries, with *P. vivax* only dominating in India and South America (Ashley *et al.*, 2006).

Plasmodium vivax

Plasmodium vivax is the second most common type of malaria, and is also associated with malaria related death, but not to the same extent as *P. falciparum*. *P. vivax* infects only reticulocytes (Weatherall and Abdalla, 1982), and therefore has a longer incubation time of 10-17 days, there is a dormant liver form (hypnozoite), which can cause subsequent infections upon reactivation (Warhurst and Williams, 1996). Correct treatment can prevent the reactivation of the hypnozoite form. Chloroquine is the recommended treatment

for *P. vivax* as resistance is low, in resistant forms alternative treatment of amodiaquine is used instead in combination with primaquine (Griffith *et al.*, 2007).

P. vivax is largely absent from West Africa as it requires the Duffy antigen to be present on erythrocytes as a receptor to facilitate entry into the cell (Luzzatto, 1979). This antigen is usually not present in natives of West Africa, therefore *P. vivax* cannot infect these people.

Plasmodium ovale

Infection with *P. ovale* is much less common than *P. falciparum* and *P. vivax*. *P. ovale* is mainly seen in Sub-Saharan Africa and in regions of islands in the Western Pacific. *P. ovale* has an incubation period of 10-17 days, and also forms hypnozoites causing incubation and reactivation (Warhurst and Williams, 1996). Resistance to drugs in *P. ovale* infections is not common and therefore treatment is usually comprised of chloroquine and primaquine (Griffith *et al.*, 2007).

P. ovale is morphologically similar to *P. vivax* but was distinguished as a separate species in 1922 (Collins and Jeffery, 2005). The main distinction between *P. ovale* and *P. vivax* is that *P. ovale* can infect cells without the presence of the Duffy antigen. The morphological distinction is that 20% of cells show a characteristic oval shape, from which the species obtained its name. All stages of the *P. ovale* erythrocytic cycle can be seen in the peripheral blood.

Since *P. ovale* was first described in 1922 (Collins and Jeffery, 2005), it has been considered that there are four different malaria species that infect humans. Genetic sub-divisions of *P. ovale* have also been proposed (Williams, 2009), leading to further complications in diagnosis.

Plasmodium malariae

P. malariae is the least common form of malaria in humans. The infection is usually benign and is commonly diagnosed as an incidental finding. Chronic infection can lead to severe complications such as nephrotic syndrome.

The slow development of *P. malariae* is different from all the other species, with slow development in both mosquitoes and humans, due to inefficient schizogony. There are less merozoites in each pre-erythrocytic schizont and also less in the erythrocytic schizonts, leading to less cells being infected during each cycle. The asexual cycle is also longer, with it taking 72 hours rather than around 48 hours for all other species. Fever occurs every fourth day because of this, and is therefore known as quartan malaria. Incubation times for *P. malariae* are also longer with a period of 18-40 days, causing a less efficient infection and a smaller likelihood of patient morbidity and mortality.

P. knowlesi

P. knowlesi, a monkey parasite, has recently been discovered to be infecting humans. The majority of cases have been reported in South-East Asia (Cox-Singh *et al.*, 2008). No training was provided for this species, which has a similar appearance to *P. malariae*, due to a lack of diagnostic material available.

2.2 Diagnosis

Diagnosis can be carried out using a number of different methods, each with their own benefits and problems. Here, each method is reviewed and relevance to routine diagnosis in laboratories in endemic countries is evaluated.

2.2.1 Clinical diagnosis

Clinical or presumptive diagnosis of malaria is carried out from the clinical symptoms alone with no diagnostic tests being carried out. This method is commonly used due to tradition and as it is the least expensive (Petti *et al.*, 2006). However, symptoms especially in the early stages of the disease are non-specific (World Health Organization and Usaid, 1999) and are seen in a number of common conditions. Symptoms seen in early infection include fever and chills, often accompanied by headaches, myalgias, arthralgias, weakness, vomiting, and diarrhoea (Centers for Disease Control and Prevention, 2008). Presumptive clinical diagnosis not only leads to misdiagnosis, but as more people are exposed to unnecessary treatment it can also promote drug resistance in the parasites (Reyburn *et al.*, 2006).

Clinical diagnosis was initially seen as the most cost effective method when treatment was inexpensive, but as drug resistance has developed more problems have emerged. With the introduction of artemisinin combination therapies the cost of treatment has increased significantly (Rafael *et al.*, 2006), meaning that presumptive treatment is no longer cost effective (Jonkman *et al.*, 1995). Partly due to the number of false positive diagnoses made in patients showing symptoms of other conditions that are misdiagnosed as malaria that would receive unnecessary treatment. The cost of chloroquine is US \$0.20 – 0.40 per course, compared to \$5-8 for artemisinin combination therapies (Economist, 2007). The specificity of clinical diagnosis is only 20-60% compared to microscopy as the reference standard (Guerin *et al.*, 2002). It can also mean the true cause of the illness remains unidentified and lead to an inability to determine the correct prevalence of disease, both of malaria and of conditions it is misdiagnosed as (Petti *et al.*, 2006). Patients that are treated with antimalarials but whose condition does not improve could either have drug resistant malaria or another condition, clinical diagnosis cannot make this distinction (Barat *et al.*, 1999). The reverse of this problem can be even more devastating when patients with malaria do not receive treatment and leading to possible increased mortality rates (Amexo *et al.*, 2004).

Different diagnostic algorithms have been shown to improve the sensitivity of diagnosis. In a study by Muhe *et al.* (1999), it was shown that the most specific diagnostic findings in malaria were pallor and splenomegaly. A combination of fever with a history of malaria or pallor or splenomegaly had a sensitivity of 80% in the high season and 65% low season. The specificity was 69% in the high season and 81% in low season (Muhe *et al.*, 1999). The WHO now recommends that laboratory diagnosis is carried out on all suspected cases (World Health Organization, 2010b)

2.2.2 Microscopic diagnosis

Using microscopy for the diagnosis of malaria is regarded as the gold standard method for the detection and identification of parasites for routine diagnosis in endemic countries (Endeshaw *et al.*, 2008). Microscopy can enable the presence of parasites, the species and parasitaemia to be determined

(Kakkilaya, 2009), at relatively low cost (Boonma *et al.*, 2007). However, microscopy is viewed as an imperfect standard (Schindler *et al.*, 2001) as the quality of the diagnosis is dependent on the skills of the microscopist. Alternatives to microscopy, as the gold standard, are sought, however, these techniques have not, as yet, completed sufficient numbers of tests to overturn microscopy as the gold standard (Ohrt, 2008). There are different opinions on the effectiveness of microscopy. The use of microscopy as the gold standard is supported by Drakeley and Reyburn, 2009; Thomson *et al.*, 2000; Moody, 2002; Wongsrichanalai *et al.*, 2007; Chotivanich *et al.*, 2007; Coleman *et al.*, 2002; Talisuna *et al.*, 2007; Kakkilaya *et al.*, 2003; Johnston *et al.*, 2006; Noedl *et al.*, 2006; Rogerson *et al.*, 2003 and Maguire *et al.*, 2006. Other papers, however, see microscopy as the imperfect gold standard (Briggs *et al.*, 2006; Wongchotigul *et al.*, 2004; Andrews *et al.*, 2005; Rakotonirina *et al.*, 2008 and Reyburn *et al.*, 2007).

Microscopic diagnosis is carried out using the blood smear, the smear can be made from a finger prick sample or a venepuncture sample. There are two different preparations that are commonly used in diagnosis, the thick and thin film.

The thin film is made by spreading the blood along the slide with another slide to create a single cell layer, allowing individual blood cells to be seen, and parasites to be detected aiding specific species diagnosis. The morphology is examined between the middle to tail of the film (Houwen, 2000) where the erythrocytes are just overlapping (Chiodini P and Moody, 1989).

The thick film is made by spreading a blood drop into an oval shape (Bruce-Chwatt L, 1993). Multiple blood cells lie on top of one another, staining causes the unfixed erythrocytes to lyse (Houwen, 2000), but not the parasites, making it easier to see parasites, in the larger volume of blood increasing the sensitivity of diagnosis. The detail in the parasites can be lost however and they can be difficult to identify without experience (Draper, 1971).

Microscopy of Giemsa stained thick and thin blood films has been carried out since the early 20th century, methods used today have changed very little from the original (Giemsa, 1904, Tangpukdee *et al.*, 2009). For the thin film, the

staining method is based on the Romanowsky stain, which uses a combination of eosin Y and methylene blue, with the use of methanol as a fixative (Houwen, 2000). A number of different variations are used including, Wrights stain, Giemsa stain, May-Grunwald Giemsa stain, Fields stain and Leishman stain. Giemsa and Field's stain (rapid or normal) are the two most common methods used. Both Wright's and Field's stain can be used in rapid diagnosis (Haditsch, 2004), however the staining is often not of the same quality as the Giemsa stain. All these stains are also used for routine blood smear staining at pH 6.8, however the pH needs to be changed to 7.2 for malaria diagnosis to allow full parasite detail to be seen (Lewis *et al.*, 2006).

The thick film should be used for identification of parasites at lower parasite densities, but not for speciation, as this is considerably more difficult than on the thin film (Moody and Chiodini, 2002). The thick film is designed to allow an increased sensitivity, however this can be affected by the preparation of the film. If the film is incorrectly prepared i.e. the blood is spread too thinly, the sensitivity can be less than the thin film (Dowling and Shute, 1966). The sensitivity can also be reduced when the blood is spread too thickly, artefacts are introduced and parasites can be difficult to see. The film can also appear to be spread too thickly when inadequate lysis of the erythrocytes occurs, usually when the film has been partially fixed or has dried too much (Chiodini P and Moody, 1989). In tropical regions, flies can also be a problem removing blood from the slides if left in the open (Shoklo Malaria Research Unit, 2002).

Examination of the blood film is carried out using the 60x or 100x oil immersion objective (Warrell and Gilles, 2002). Each different species of malaria has a different appearance on the blood film. Different species also show different stages of infection on the blood film. Schizonts are rare in *P. falciparum* infection and are only present in severe infection, whereas *P. malariae* infections normally show all stages (Choudhury and Ghosh, 1985). The quality of diagnosis can be affected, both by the individual's experience and the quality of microscope that they have access to (Opoku-Okrah *et al.*, 2000).

The quality of diagnosis by microscopy depends on the facilities available but also on the training of the staff. Electricity supplies in rural areas can be

unreliable, restricting the equipment that is available to be used in the laboratories. Microscopes in these laboratories may be old and not be of an adequate quality for diagnosis. Two microscopes were being used which had no focusing ability (Mundy *et al.*, 2000). Variations between different microscopes have been shown to influence results of tuberculosis testing (Lumb *et al.*, 2006). The microscope has also been shown to be of an influence in malaria diagnosis (Kilian *et al.*, 2000). Training can be problematic; there may be no one with adequate experience to do the teaching and monitor performance. Often rural laboratories will have one or two members of staff, some with little training. There is a lack of recognition of quality assurance in these sites and little regulation, resulting in a lack of promotion of improvements in results (Petti *et al.*, 2006).

2.3 Variables affecting the accuracy of malaria diagnosis by microscopy

The accuracy of diagnosis of malaria is variable between different locations and different individuals. The accuracy of diagnosis has been shown by various investigators to be influenced by

- Staining method (Mendiratta *et al.*, 2006)
- Thick or thin film (Mendiratta *et al.*, 2006, Ohrt *et al.*, 2008)
- Method for calculation of parasitaemia (O'meara *et al.*, 2006b)
- Variation between slides (O'meara *et al.*, 2005)
- Artefacts and stain debris (Milne *et al.*, 1994)
- Species of malaria present (Milne *et al.*, 1994)
- Equipment available (Mundy *et al.*, 2000)
- Reader technique (O'meara *et al.*, 2006a)
- Training improving diagnosis (Ngasala *et al.*, 2008)

Mendiratta *et al* (2006) compared blood film microscopy using the Leishman stain on thick and thin blood films to Field's stain, a modified acridine orange

stain and the Paracheck Pf antigen kit (HRP 2). Mendiratta compared 443 smears evaluated by two microscopists to determine the presence or absence of malaria. Field's stain detected only 28 out of the 81 cases detected by the Leishman stain. Problems have been reported with the Field's stain film occasionally washing off of the slide (Lema *et al.*, 1999). Leishman stain is not commonly used in the UK as Giemsa is used in other staining techniques and is quicker and easier to use for batched samples (Dowling and Shute, 1966).

Ohrt *et al* (2008) has shown differences between thin and thick films stained with the same stain. Specified criteria were used to avoid variation in slide preparation, however, the thick and thin blood film were made on the same slide. This risks the thick film being fixed preventing cell lysis (Cheesbrough, 2005). Thick and thin blood films on separate slides help to aid correct and accurate diagnosis (Draper, 1971), however one slide reduces costs and is easier for staff (Cheesbrough, 2005). Ohrt's study involved the independent reading of thirty-six thick and thin films, eight microscopists read the thick films and five read the thin films, as part of an investigation into training of microscopists. Only one person was used to read both the thick and thin films. The study showed considerable disagreement between microscopists; on the thin film there was 53% disagreement, with 42% at variance over for positivity or negativity, and 58% due to species determination. This paper does not compare the sensitivity of the thick and the thin film, but the individual's interpretation.

O'Meara *et al*, (2006b) showed differences in the parasitaemia evaluation using different counting methods. The grid method (counting cells within a grid) was compared to the RBC method on thin films and the WBC method on thick films. The study was well designed with eight microscopists taking part, receiving training for a week in the techniques prior to sample analysis. Densities recorded by the grid method were significantly lower than using the WBC method. Overestimation of parasitaemia was seen at higher densities and an underestimation at the lower concentrations using all the methods. One microscopist's results were discrepant and their results were excluded. This weakened the experimental design, but also raised concerns over the consistency of microscopists preceding training. The WBC method used

accurate white cell counts to determine the density, aiming to ensure that the only discrepancy was between readers. For thick films, the grid method gave discrepancies, there were no discrepancies seen on the thin film, parasite loss during staining of the thick film may have accounted for this (Dowling and Shute, 1966).

An earlier paper by O'Meara *et al* (2005) compared different individuals looking at the same slide, and also one individual looking at different slides from the same patient, and then asked them to make a diagnosis and calculate the parasitaemia. To minimise equipment variation between readings, microscopists were supplied with identical microscopes. The findings suggested that the discrepancies between the readers decreased as the parasite level increased, mainly due to different techniques in parasitaemia counting and the parasite level. This could suggest that with further training there would be more reliability in the results given. 242 slides produced from a single patient sample were examined by slide readers and an expert microscopist. The expert microscopist however, was not the same for every slide. Discrepancies between the slides were significantly lower than between readers, with lower densities showing the greatest differences.

Milne *et al* (1994) carried out a comparative study of samples submitted to two reference laboratories. There were 17 *P. ovale* infections of which only five (29.4%) were diagnosed correctly, 162 (77.1%) single infection cases (excluding *P. ovale* cases) were correctly diagnosed. Only one of six mixed infections was correctly diagnosed. Sequestrene effects were seen in 85% of samples due to prolonged storage in EDTA. There were 104 technical faults from 82 specimens, acidic pH was the most common problem, occurring in 46 specimens. The correct diagnosis was given in 12 out of 15 cases with high platelet counts, with one laboratory reporting a false positive *P. falciparum* infection. There was no significant correlation between technical faults, the number of platelets or an incorrect diagnosis. As only samples referred to the reference laboratory were analysed, there is a bias in the samples present, and false negative samples would be missed.

Mundy *et al* (2000), carried out an observational study of microscope condition in Malawi. One questionnaire was distributed in each district, there were a total of 90 microscopes examined, averaging 10 per district (range 3-24) of which only 50% were in a good condition; 13% of the microscopes were unusable, 22% required attention. This indicates that the microscope quality is poor and with poor microscopes accurate diagnosis is made even more difficult (Opoku-Okrah *et al.*, 2000). The questionnaire was only distributed centrally; there may have been more microscopes on site that staff were unaware of. Assessment as to whether the microscope was in a usable condition has been made by the laboratory involved and this judgement may change throughout the region.

O' Meara *et al* (2006a), assessed the sources of variation in reader technique. The interpretation by 27 microscopists of 895 slides collected from 35 donors was monitored. Samples were stained in batches to avoid any cross contamination. The parasitaemia was reported as the absolute number of parasites counted on the examined area. Variability between readers included interpretation and handling technique. Technique variations were mainly sampling errors in the calculation of parasitaemia, with different individuals counting different numbers of cells. This varied from 8600 WBC to three WBCs and 150,000 RBC to 400 RBC. The parasitaemia calculations were more accurate on the thick film. There were however fewer parasites counted on the thick film, which could be due to parasites washing off the slide as reported in 1966 (Dowling and Shute). It is difficult to see from these results the performance of individual's, to determine whether variability was due to a few individuals or generalised across the group and relates to the analytical performance of the laboratory.

Ngasala *et al* (2008) investigated the accuracy of diagnosis in 16 laboratories in Tanzania, three were in health centres and the rest in dispensaries. These were split into three groups, Arm I received training on microscopy and clinical diagnosis, Arm II to receive training on clinical diagnosis and Arm III received no training. Significantly less antimalarial drugs were prescribed in Arm I compared to any other, less than 50% of the other groups, 76.7% of antimalarial drugs were correctly prescribed. 936 blood slides were re-examined at the reference

centre, 607 (65%) agreed, 269 true positives and 338 true negatives. Overall sensitivity was 74.5%, specificity 59%, positive predictive value (PPV) 53.4% and negative predictive value (NPV) 78.6%, higher sensitivity was shown at higher parasite densities. 11.3% of patients with a negative blood smear had been prescribed antimalarials. As there was poor accuracy in diagnosis, to prove that the training had a true effect on diagnosis, pre and post analysis results should have been analysed at each location.

2.4 Other methods of malarial diagnosis

Due to problems in accuracy identified by previous studies discussed above, alternative less subjective methods are always being sought. The current methods that are being used alongside microscopic diagnosis are

- Rapid diagnostic tests (RDTs)
- Molecular diagnosis
- Quantitative Buffy Coat method
- Antimalarial antigen detection
- Detection of malarial pigment
- Dark field microscopy

2.4.1 Rapid diagnostic tests

Rapid diagnostic tests give a rapid result in as little as 15 minutes, from a finger prick sample, and require little training to give a positive or negative result.

RDTs mainly detect three antigens, the histidine rich protein 2 (HRP-2), parasite lactate dehydrogenase (pLDH) and *Plasmodium* aldolase antigens (Moody and Chiodini, 2002, Kakkilaya, 2003).

HRP-2 is expressed only by *P. falciparum*, found in all stages of infection as it is expressed on the membrane surface of the red cell (Kakkilaya, 2003). The protein is water-soluble and has been detected for up to 28 days after the start of antimalarial therapy. Humar *et al* (1997) showed that 27% of patients still had a positive test result after 28 days. Swarthout *et al* (2007) also showed

prolonged presence of the antigen up to 35 days after initial treatment.

pLDH is expressed by all four of the *Plasmodium* species by all stages of live parasites. The soluble glycolytic protein is released by the infected cell as well as being present within the cell (Kakkilaya, 2003).

Plasmodium aldolase is also expressed by all *Plasmodium* species. This an enzyme of the glycolytic pathway (Kakkilaya, 2003), used to help detect non-falciparum infections. Aldolase is usually used in combination with HRP-2 to allow for the detection of non-falciparum infections (Wongsrichanalai *et al.*, 2007). However, using these kits, mixed infections cannot be ruled out when *P. falciparum* is present.

The sensitivity and specificity of these tests is said to be approaching that of microscopy (i.e. 100-200 parasites/μl). The sensitivity of the kits is dependent on the parasitaemia of the case. When parasites were present at <100 parasites/μl the sensitivity fell to 53.9% (Murray and Bennett, 2009). Below 1000 parasites/μl the sensitivity of *P. vivax* the sensitivity falls to 47.4% from 81% for those with more than 1000 parasites/μl. Below 100 parasites/μl the sensitivity for *P. vivax* was 6.2% in Murray's experiments (Murray and Bennett, 2009).

Other considerations when using RDTs should be taken into account when making a diagnosis. RDTs do not allow quantification of parasitaemia, meaning that a low parasite density will receive the same treatment as a high parasitaemia case. In very high parasite density cases the kit may appear to be negative, as there is too much antigen present for the enzyme to react with (Van Den Ende *et al.*, 1998). High storage temperatures can also cause inactivation of the strips, (Jorgensen *et al.*, 2006).

RDTs are also more expensive in comparison to blood films (Wongsrichanalai *et al.*, 2007), and in many regions had not been used in preference. The cost of each test varies by location, Mosha *et al* (2010) reported malaria microscopy as costing US \$0.27, RDT \$0.75 and ACT treatment as \$0.95. Batwala *et al* (2010).

Rapid diagnostic kits are now being introduced into endemic areas, where some problems have been encountered. Due to storage conditions the strips

can become inactivated by high heat, but there is no way of knowing if the strip has been affected, leading to false negatives. Other antigens within the body have also been shown to react with the HRP-2; rheumatoid factor can cross react with the system, also leading to false positive results. Whilst the pLDH test gives a positive or negative result, due to the lack of species identification, microscopy will still be required to determine the species present. The WHO recommends the use of RDTs if microscopic diagnosis is not available (World Health Organization, 2006)

2.4.2 Molecular diagnosis

Molecular diagnosis is carried out in malaria diagnosis to determine the species present but can also be used quantitatively. Molecular techniques for malaria diagnosis, is based upon the identification of the small subunit of ribosomal RNA (ssrRNA) of *Plasmodium* (Singh *et al.*, 1996). This can be used for the detection of all species, as different sequences are present for each species. Polymerase chain reaction (PCR) can be carried out in two ways, nested PCR can be used with four independent reactions for species determination, or direct PCR can be used for the detection of *P. falciparum* (Rubio *et al.*, 1999). The nested PCR can be carried out by different mechanisms, the standard is to use a semi-nested multiple PCR to amplify the ssrRNA using a single reaction, four species specific primers and a universal *Plasmodium* primer are used for the second amplification (Rubio *et al.*, 1999).

Some of the conditions which are used for the initial amplification of the DNA differ for the different species. Johnston (Johnston *et al.*, 2006) reported using the same denaturing temperature of 94°C for each species, with variations in the annealing temperature and time for each species. This further complicates the procedure, making it more difficult to integrate into routine practice. Results showed an increased sensitivity compared to microscopy, with a sensitivity of up to 99.5%. Sensitivity for *P. falciparum* has been reported down to 0.004 parasites / µl (Elsayed *et al.*, 2006), a comparison of the different sensitivities achieved is given by Bourgeois (Bourgeois *et al.*, 2009). The sensitivity of microscopy is around 20 parasites / µl (Jonkman *et al.*, 1995).

Molecular diagnosis is currently used only to confirm microscopy findings, as

results can take a day to be obtained (Johnston *et al.*, 2006), causing significant delays in patient treatment. PCR has the capacity to detect very low levels of parasitaemia, and is seen as being the most sensitive and specific method, but currently is too expensive for routine use in endemic regions, and requires not only a lot of complex techniques but also the use of large numbers of highly specialised reagents (Hanscheid and Grobusch, 2002). The current development of automated methods using real time PCR (Farcas *et al.*, 2004) aim to decrease the complexity of diagnosis to make it suitable for routine diagnosis, and also increase the speed at which diagnosis can be made.

In endemic regions molecular diagnosis is not currently suitable for routine diagnosis. However, trials have been carried out in endemic countries. Mens (2007) investigated the use of PCR in rural Kenya and urban Tanzania. This investigation showed that in rural areas microscopy in combination with RDTs are the most accurate, due to a lack of facilities to provide PCR based tests. In the more developed hospital laboratories molecular diagnosis can be used, providing that the adequate skills are available.

2.4.3 Quantitative Buffy Coat

The Quantitative Buffy Coat (QBC) method involves the staining of nuclear material with acridine orange stain. The technique is a variation of fluorescence microscopy, in which the cells are centrifuged in a capillary tube prior to staining enabling separation of cells by mass (Chotivanich *et al.*, 2007). Acridine orange stains the nucleic acid-containing cells (Makler *et al.*, 1998), highlighting white blood cells and parasites, which can then be identified using UV light. The nucleus of the parasite stains bright green, with the cytoplasm appearing yellow-orange (Chotivanich *et al.*, 2007).

The main cost with this technique is the fluorescent microscope, however it may be used for other laboratory techniques, to justify the cost of fluorescence microscopy. The capillary tubes (haematocrit tubes) needed for the test are expensive, and there have been difficulties in species identification (Adeoye and Nga, 2007). The capillary tubes are also difficult to store and therefore can be read only once, which may lead to difficulties in cases that need to be referred back to, or in performance monitoring.

The sensitivity of the method is disputed between different studies that have been carried out, but is generally said to be similar to the thick film (Moody and Chiodini, 2002). The sensitivity with <100 parasites / μ l has been reported to be between 41.1% and 93% (Delacollett and Vanderstuyft, 1994), the specificity however is affected by the species. Hakim *et al* (1993) reported the specificity for *P. vivax* to be around 52%; in contrast the specificity for *P. falciparum* was reported at around 93%.

Clendennen *et al* (1995) compared the sensitivity and specificity of QBC method versus Giemsa thick blood films in a group of inexperienced laboratory technicians. The sensitivity achieved with QBC was 75% compared with 84% for Giemsa stained thick films. However, the specificity was improved with the QBC method with a specificity of 84% versus 76%. The authors believed that improved sensitivity would be achieved with experience.

There are problems with the disposal of acridine orange as it is considered hazardous (Moody and Chiodini, 2002). However, the method is deemed to be suitable for malaria diagnosis (Moody and Chiodini, 2002), either alongside Giemsa thick and thin blood smears or alone.

2.4.4 Malarial antibody detection

The serological detection of antibodies against the asexual stages of malaria, is usually carried out using Immunofluorescence antibody testing (IFA) (Tangpukdee *et al.*, 2009). A wide range of antibodies are produced, specifically against the malaria antigens. The plasmodium antibodies can be specific to the stage of infection as well as species present (Castelli and Carosi, 1997). Antibodies can persist for months or years in the case of individuals who are constantly exposed for example, making the method inadequate for diagnosis in endemic regions.

The IFA testing can be used to specifically detect either IgG or IgM antibodies. If the antibody titre is below 1:20 the diagnosis is unconfirmed and probably negative, above 1:20 is positive and above 1:200 probably represents a recent infection (Chotivanich *et al.*, 2007).

The test is simple and highly sensitive and specific, but cannot be used for

routine diagnosis due to the time taken for antibodies to be produced and therefore the result cannot be detected for some days after initial infection (Warrell and Gilles, 2002). This limits the use of the method to retrospective diagnosis in those who have already been treated (Hanscheid, 1999). This method is also therefore of limited use in endemic regions where most individuals have malaria antibodies present.

2.4.5 Automated Detection of malarial pigment

This is a relatively new method using automated analysers and software programmes to detect the malarial pigment in white blood cells using flow cytometry (Hanscheid *et al.*, 2001). Detection of the malaria pigment in leukocytes is by the use of depolarised laser light, Volume Conductivity and Scatter techniques (Tangpukdee *et al.*, 2009). The method is still in the developmental stages (Briggs *et al.*, 2006).

There have been a number of studies carried out in this area by the instrument manufacturers, Abbott Cell-Dyn 3500 and 4000 have been the most widely published. Initial studies were carried out using the Cell-Dyn 3500 to detect the presence of malaria pigment (haemozoin) within leukocytes (Hanscheid *et al.*, 2001). Compared to microscopy a sensitivity of 95% was achieved and a specificity of 88%. Five false positive cases were reported, in those who had previously had malaria.

The Cell-Dyn 3500 was also used to detect the presence of malaria using polarised laser light, this had a sensitivity of 72% and a specificity of 96% (Mendelow *et al.*, 1999). The Cell-Dyn 4000 has since been shown to have increased specificity at 98% (Padial *et al.*, 2005).

Beckman Coulter has also trialled this system on the LH 750 (Briggs *et al.*, 2006) and the Gen.S (Fourcade *et al.*, 2004). The LH 750 demonstrated a sensitivity of 98% and specificity of 94% (Briggs *et al.*, 2006). The Gen.S system using combined parameters gave a sensitivity of 97% and specificity of 83% (Fourcade *et al.*, 2004).

For this method to be applicable in the endemic regions, automated analysis is required, and this is generally not available in most endemic regions, however

another confirmatory test would be required.

2.4.6 Laser desorption mass spectrometry

Laser desorption time-of-flight mass spectrometry has been used in trials. It detects heme that is concentrated by the parasites. The process begins with RBC lysis to free the parasite; the parasite is then lysed to detect the heme within it. There is potential to use the method in the field but is considerably more expensive and complex than other methods (Demirev *et al.*, 2002).

Parasites can be detected at parasitaemias as low as ten parasites/ μl .

2.4.7 Dark field microscopy

By using wet preparations of blood films both thick and thin unstained, using the dark field setup on the microscope, parasites can be viewed. There are similar levels of training required as for standard Giemsa microscopy, and detail is difficult to see. The parasites are bright patches in the dark field (Jamjoom, 1983), however there are no reports of comparisons to the other features seen.

2.5 The cost of misdiagnosis

Misdiagnosis of malaria affects patient outcomes and increases the economic burden of the disease (Amexo *et al* 2004). False positive diagnoses lead to unnecessary treatment that, in turn, may lead to drug resistance and unnecessary expenditure. In attempting to determine the economic cost of misdiagnosis in North-Eastern Tanzania, Mosha *et al* (2010) found that misdiagnosis occurs at a rate of 45% in some geographical areas and that costs could be reduced by up to 15% by lowering the number of false positive diagnoses. In another study in Sudan, A-Elgayoum *et al*, (2009) reported a false positive diagnosis rate of 75.6%. These authors estimated the cost of diagnosis and treatment of malaria to be \$100 million, whereas they calculated the true cost should be \$14 million. It was also determined that 43% of the general practitioners (GPs) lacked the clinical experience in recognition of malaria symptoms.

2.6 Conclusions

Microscopy is still regarded as the most reliable method available for diagnosis of malaria in endemic countries. As new methods are developed sensitivity and specificity should improve, but problems with false negative results, lack of ability to monitor treatment and complexity of techniques, limit the current practical applications of these novel techniques. Molecular methods, alongside microscopy, are becoming increasingly regarded as the gold standard method in the UK (Bailey *et al*, 2005).

Chapter 3: Generation of the microscopic images to be used for the assessment of competency of diagnosis of malaria

Virtual microscopy has been developed over the last ten years, with many possible uses including education, training and quality assessment (Lundin *et al.*, 2004). The virtual microscope can be used in all pathology disciplines, with most research being carried out in histopathology. During the development of virtual microscopy a number of obstacles have been discovered, the biggest of which is storage capacity of images (Albe and Fierz, 2005).

A virtual microscope is an interactive tool that can be used to visualise a digitised microscope slide (Albe and Fierz, 2005). The area of the slide digitised depends on the amount required to achieve an accurate diagnosis, the magnification required and the size of the final file. The technology is usually used for a static image, however it can also be used for the transmission of live real time images across the Internet (Lundin *et al.*, 2004).

Virtual microscopy, can be delivered in many forms, single images can be used to highlight features and guide on approaches to examination. Large scale stitched images, virtual slides (Burthem *et al.*, 2005) are used to give a representation of diagnosis in the laboratory, giving a healthcare scientist an opportunity to test their skills, perhaps passing a competency test before they are permitted to undertake diagnosis on a patient sample.

3.1 Application of virtual microscopy

Gu and Ogilvie (2005) report that microscopy was first introduced into medical training in Edinburgh, Scotland in the 1830's. By 1990 computerised assisted learning was used alongside microscopy for training. Heidger *et al* (2002) have proposed that due to the recent changes in the training of medical staff, there is less time on the curriculum for teaching practical pathology and therefore not enough time to provide adequate microscopic training. There have been many studies carried out on the examination of histological slides using virtual microscopy and the implementation of this technique in medical and healthcare

science education (Fontelo *et al*, 2009; Rossier, 2009; Koch *et al* 2009, Lundin *et al*, 2004; Burton, 2005; Treanor, 2009; Dee, 2009; Heidger *et al*, 2002; Kumar *et al*, 2004). .

Heidger *et al* (2002) report on the use of virtual microscopy in the teaching of histology at University of Iowa College of Medicine, where the virtual microscope was provided alongside conventional microscopy, initially as the laboratory session introduction. The light microscope was used to confirm findings, reviewing material after the session and for revision. Virtual microscopy was also used for examination, with students achieving similar results to previous years when traditional microscopy was employed. The new procedures were rated highly by students. An alternative method was employed by Deniz and Cakir (2006) who generated prototype software on CD for use in histology education, which was trialled with ten students. They explored the design of a formative environment for computer-assisted learning. Student comments were reported and future design modifications were discussed. An alternative method was developed by Goubran and Vinjamury (2007) who developed an interactive atlas for histology. This method was used in a controlled trial, with one group not receiving access to the atlas. The final assessment results of the group exposed to the atlas were significantly higher than the not exposed group.

In addition to these studies Dewhurst *et al* (1994) experimented with computer assisted learning techniques. Some of the computer-assisted learning was designed to simulate laboratory experiments in histology, however not necessarily microscopy. The knowledge gained compared to standard teaching methods was shown to be equivalent, but the costs were considerably lower for the computer-assisted learning.

There are very few haematological studies carried out for the use of the virtual microscope. However, a few studies have been carried out in teaching and learning for parasitology. Gunn and Pitt (2003) used computer-assisted learning for the teaching of one part of the parasitology curriculum. In the first year, the computer-assisted learning was delivered alongside the curriculum. In the second year, the online training was provided alone. In the year subsequent to

this, the exam results for this section were considerably lower, although they reported spending more time on the training than other years.

Virtual microscopy can be used to compare an individual microscopists results on the virtual image to the glass slide and can also be used to monitor an individual's performance over time. Furness (2007), using renal sections, compared the accuracy of diagnosis of the virtual microscope versus the conventional light microscope. There was no significant difference between the two groups, however the virtual slides took slightly longer to examine. Only a small proportion of participants completed the virtual microscope images, diagnosis was submitted for six out of the 12 cases by 27% of participants. The conclusions that can be drawn are therefore limited.

In haematology, there are a number of websites offering limited area images in a simple atlas format. The larger format images have so far been used in competence assessment and external quality assurance. UK NEQAS (H) has developed a continuing professional development scheme that uses a virtual microscope (Hutchinson *et al.*, 2005, Burthem *et al.*, 2005). Initial trials involved the use of single visual field images showing key features in a blood film. Participants were asked to identify abnormal features and choose those that are diagnostic, making a diagnosis where possible. Following this a quick time image was used to allow the user to move around the image. Recently the team has collaborated with a software company to provide a CPD scheme with a virtual microscope that allows use of multiple magnifications as well as movement around the specimen. The basic virtual microscopy system was developed as described by Costello *et al* (2003).

A similar scheme has also been trialled by the Royal College of Pathologists Australia Quality Assurance Programmes Pty Limited (Intan *et al.*, 2009). Three images taken using the Aperio ScanScope slide scanner were provided online for individuals to make a diagnosis, these results were compared with the results of the specimens on glass slides from which the images were taken. The diagnosis was similar on the first two of the cases, however large differences were seen on the last case. The slide appears to be identical in size to the original. The resolution of the scanned image has been shown to be poor for

small features present in white cells (Sibanda *et al.*, 2009), which could explain these difficulties.

The use of virtual microscopy in telemedicine has been described in a number of situations. Telemedicine in these circumstances is the use of a virtual microscope image to be used for diagnosis at another location or for consultation on diagnosis. There is potential for this technology to be used in remote diagnosis in developing countries where the expertise is not available on site. Fontelo *et al* (2005) investigated the use of virtual microscopy in medical education and telemedicine in these regions. They concluded that the area of the image chosen by the microscopist affected the results, however all the results came to the same diagnosis as those resulting from slide examination.

Murray *et al* (2006) used email and live transmission over the Internet at different speeds, to send different images to readers. 221 images were analysed for the presence or absence of malaria and also for speciation. When images were deemed to be of sufficient quality the presence of parasites were determined in 98% of cases. Of those speciated 86% was carried out correctly, with a higher proportion of correct results for emailed compared to the live transmitted images. Participants were confident to treat malaria when truly present in 62% of cases, and withdraw treatment in 36% of negative cases.

A similar trial was carried out in Africa by the Réseau Afrique Francophone de Télémédecine (RAFT) project (Bagayoko *et al.*, 2006). The physician in this case was based in a reference centre. Images were sent remotely and via the Internet, to the physician to confirm diagnosis. The Internet was also used for training, using digital libraries to improve diagnosis. The system has now been expanded to other regions of the country.

Linder *et al* (2008) have produced a virtual microscope for use in quality assurance for parasitology. The system was produced to be viewed over the Internet, with the whole slide being visible and also a zoom tool to which allows higher magnification to be seen. The system does not provide any feedback, but does provide a virtual microscope for a range of examples.

3.1.1 Advantages and disadvantages of virtual microscopy

Advantages

- Allows image to be accessed at any time or place
- Identical images can be viewed by a number of individuals at the same time
- Image can be annotated to provide feedback
- Useful for rare cases where there would not be enough glass slides to be used for educational or quality assessment purposes.
- Images are cheap and easy to distribute (Hutchinson *et al.*, 2005)

Disadvantages

- Does not allow experience of using a microscope
- Viewing techniques are not the same as using a microscope (Hutchinson *et al.*, 2005)
- Optimal viewing requires high quality equipment
- Image quality will be affected by equipment used
- Require fast internet access
- Representative part of blood film must be used
- Access to a fast reliable computer is required

The following sections explain how the images for competency assessment were developed and delivered throughout the project.

3.2 Sourcing malaria samples for imaging

3.2.1 Introduction

Before digitisation of specimens could occur, the specimens on glass slides for the initial and final assessment had to be collected. At least 80 slides would be required to allow the assessment to be successful. Some specimens would also

only be used for gallery images as these were present at very high parasite density and were not suitable for detecting whether parasites were present.

Cases were sought to fit the following criteria

- All malaria species
- Species distribution similar to that found in practice
- Different parasite densities
- Presence of artefacts
- Different staining methods
- Thick or thin blood films

The specimens were chosen to reflect those cases seen in routine diagnosis. There were more *P. falciparum* cases chosen than the others as this reflects the routine laboratory workload.

3.2.2 Method

To obtain specimens participants involved in the project were asked if they were able to provide specimens to enable the images used to be as close to those used for routine diagnosis as possible.

Despite ethical approval being sought from three locations and a patient consent form being drawn up, no specimens were received from overseas laboratories, as there was not significant incentive for the extra workload involved.

Glass slide specimens were obtained from The Hospital of Tropical Diseases, London and UK NEQAS for General Haematology. These samples were obtained for external quality assessment purposes and therefore under the Human Tissue Act regulations do not require ethical approval for this purpose. The UK NEQAS samples were distributed as part of the blood film parasites scheme over the last ten years. For each of these specimens there is molecular

confirmation of the microscopic diagnosis made. The UK NEQAS slides also have consensus diagnosis and results from over 400 individuals.

3.2.3 Results and discussion

Of the 80 cases used in the project, 20 were obtained from The Hospital of Tropical Diseases, with the remainder being from UK NEQAS for Parasitology. All cases used had the diagnosis confirmed by PCR.

3.3 Generating images of blood films for virtual microscopy

3.3.1 Introduction

To provide a virtual microscopy system to be used for competency assessment, the images provided were required to be of a high quality. The images provided should be the best possible, which then enables individuals to find the same type of cells on their own microscope, in the samples they see on a daily basis.

The resolution of the microscope and the camera combined has to be good enough to allow small differences between cells to be detected. For haematology for example, a resolution of 0.2 – 0.5 μm is required for the identification of neutrophil granules (Burthem, et al., 2005). Without this resolution identification could be difficult and stippling in parasites would be missed, making species determination very difficult.

3.3.2 Methods

Microscope used for generating virtual images

Microscopes for generating virtual images are available from a number of manufacturers, these are either fully automated (Aperio ScanScope), semi-automated (Zeiss AxioImager M1) or manual (Nikon DS Fi L₂ digital camera and Eclipse microscope). For this project mosaic images were generated from consecutive overlapping fields. The criteria for choosing the virtual microscope system were based on automation and the image quality.

For research purposes as high a resolution as possible was required to give an experience as close as possible to the real microscope. Manchester Royal Infirmary conducted extensive trials in 2006 with different systems to find the best system for use with a blood smear sample. For these reasons the Zeiss AxioImager M1 microscope was chosen for imaging (figure 3.1). The digital camera option chosen was the HRc (412-312) with 1.2, 5 and 12 MP resolution available. The x63 lens (Plan APO CHROMAT 1.4 oil, $\infty/0.17$) was used to give a high quality image.

The microscope was connected to the attached PC via a universal serial bus (USB) two connection to enable the fast transmission of the image from the microscope onto the computer screen.

The semi-automated system has a mechanical stage, which was motorised and controlled to automatically scan slides. The stage also had an autofocus system. The whole system was controlled by Zeiss proprietary software (Axiovision 4.7).



Figure 3.1: The Zeiss Axio Imager M1 microscope

Generating images

The AxioVision software allows both single image and stitched image generation. There were three different settings for taking images using the AxioVision software. A “Live” window allowed the acquisition of single images, an “Acquisition” window allowed guided manual selection of fields for capture as

part of a stitched image, and the “Mosaic acquisition” carried out an automatic stitch using auto focusing if required. The second of these, the “Acquisition” window is illustrated in figure 3.2.

A number of variables in image capture were examined. Initial settings are described here and these were further developed throughout the project.

Prior to taking an image, settings have to be made in the microscope, control software and the camera.

Microscope settings

- Optimise condenser
- Mount slide and initially focus
- Locate image area to be acquired
- Note stage-starting position.
- The microscope screen displays x, y and z values.
- Focus using the 63X oil immersion lens

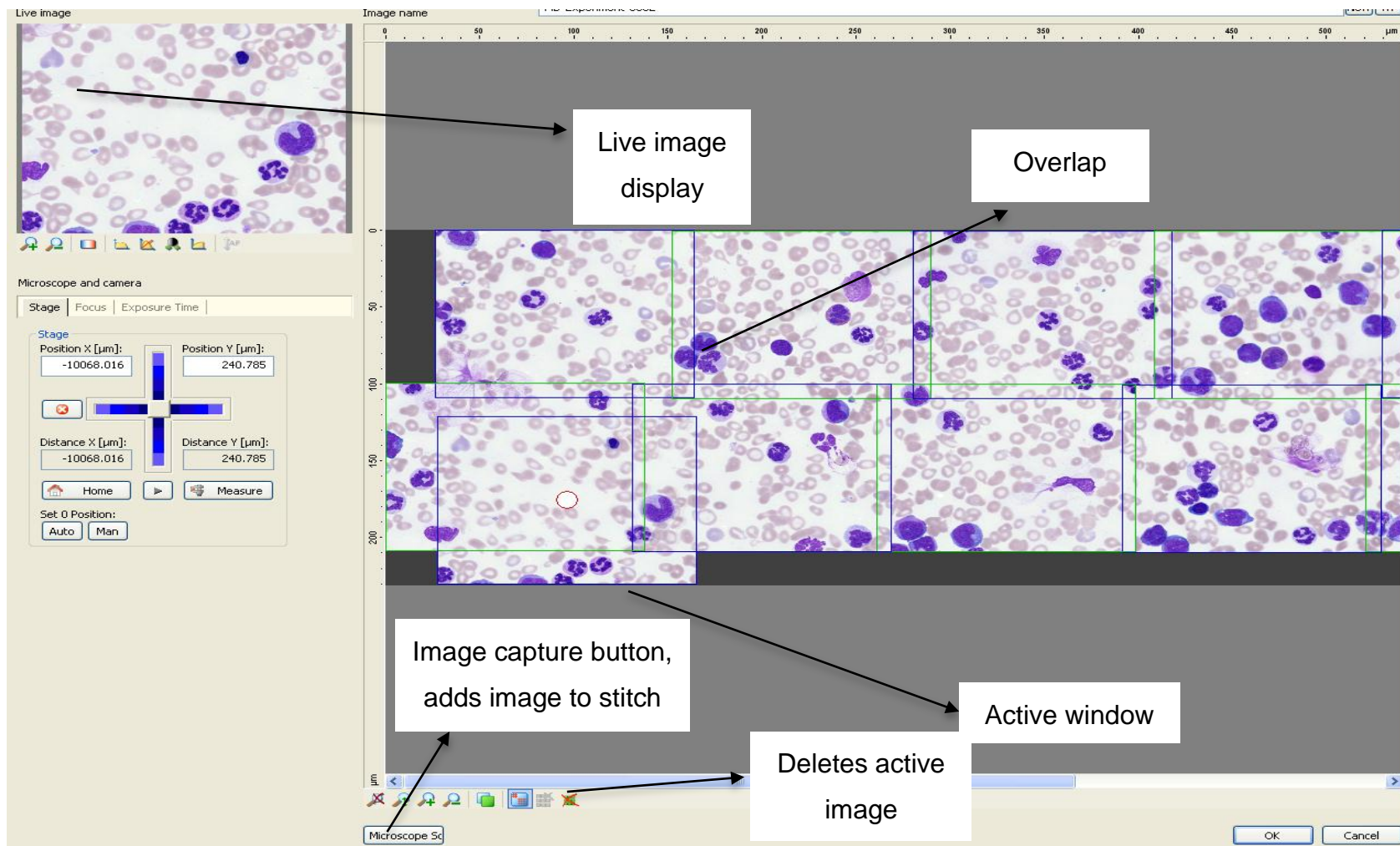


Figure 3.2: Generating a stitched image in Axiovision

Camera settings

- Resolution

Resolution was set at 3900 by 3090 pixels (12 MPx)
(figure 3.3)

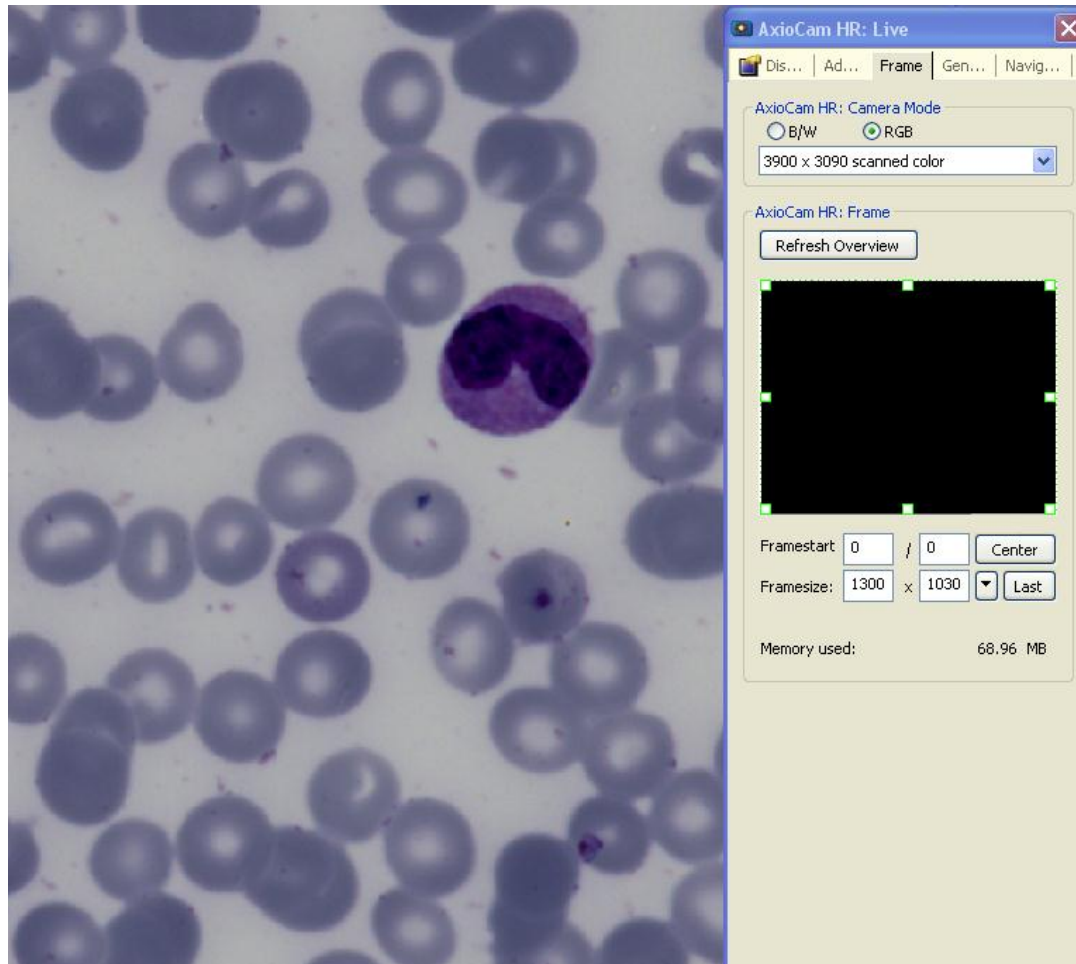


Figure 3.3: Adjusting the resolution and frame size on the AxioImager microscope

- Frame size

The frame size is set via the Live properties display on the frame tab (figure 3.3)

The size of the mosaic image chosen for this project was 40 microscope fields, this consistency prevented a bias between the different slides used. 40 images were chosen as this was close to the maximum size the computer

could process at 12 MPx setting, with each field being over 30 Mb, making the stitch over one Gb.

Automated stitching

Automated stitching used the “Mosaic acquisition” window within AxioVision. Initially the area to be photographed was chosen and mapped on the screen (figure 3.4). To allow the images to be joined together accurately, there needed to be an overlap of more than 10%, to allow the stitching software to correctly align images. Once these settings had been inputted, the focussing options were chosen. The focus can either be turned off, so the slide will be taken in the same focus as was present at starting the acquisition or focus correction chosen, with focus points chosen manually around the mapped area or autofocus could be carried out on every tile or every other tile.

Advantages

- Quick to set up and create stitched image
- Once area is chosen minimal input is required
- Ideal for images with little variation in focus plane

Disadvantages

- Autofocus may focus on the wrong plane within the image
- Vibration during imaging can cause focus to be lost during acquisition
- Autofocusing can take a long while and requires a fast stable computer system to work effectively

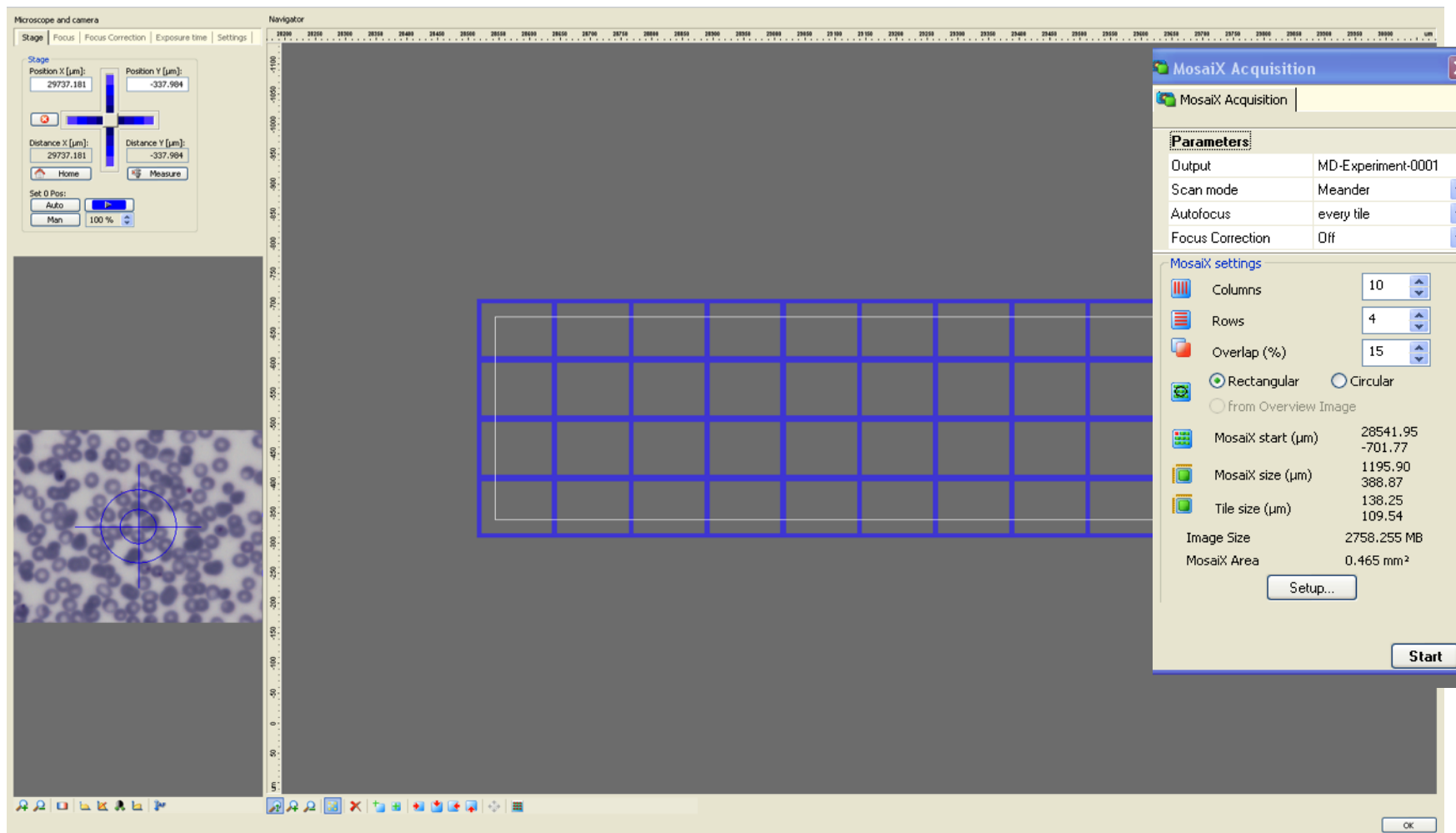


Figure 3.4: Automated stitching, planning out the area to be stitched and choosing the overlap in Axiovision

The autofocus feature could determine the incorrect focus plane based on cellular detail, which is not necessarily in the plane of interest.

Manual stitching

Manual stitching was carried out using the “Acquisition” window with each image being chosen individually shown in figures 3.2 and 3.5. Each image was chosen by moving the acquisition frame or the stage to where it was required. The overlap between the images must be sufficient to provide the software with enough data to allow accurate stitching. The overlap between ten and 15% was required for either of the methods described below. Thick films required a larger overlap as the background was less consistent and often not as densely packed as the thin film.

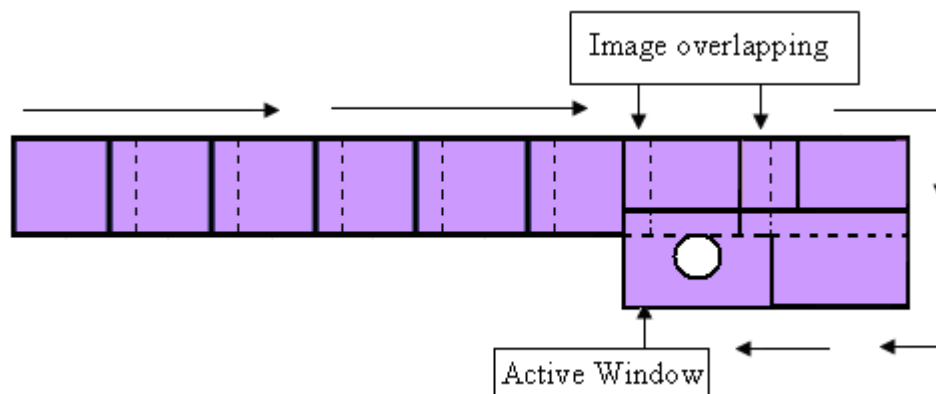


Figure 3.5: Producing a stitched image with overlap, the current active window is highlighted

Advantages

- Allows control of each image taken
- Produces a higher quality image
- Gives better representation of the images taken

Disadvantages

- Time consuming
- Some focusing problems may still be present

Single images e.g. of key features can be captured from the “Live” window, but cannot be used to generate stitches. The Live image allowed increased magnification and focusing of the image, and could also be used in combination with the live properties window to set up the microscope.

Image files were either saved individually to be stitched later or just the final stitch produced in the AxioVision software. To save the entire stitch “Save as” was used. To save all the individual images “Save all” was used. File formats were chosen from ZVI (Zeiss Raw format), JPEG and TIFF formats.

3.3.3 Results and discussion

Camera settings

Resolution

To assess the range of zooming possible, trials were run testing low and high magnification of the acquired image at each camera resolution setting.

Figure 3.6 a and b shows the image taken of *P. falciparum* gametocyte with the 1.2 MPx camera, b shows the isolation of single cell

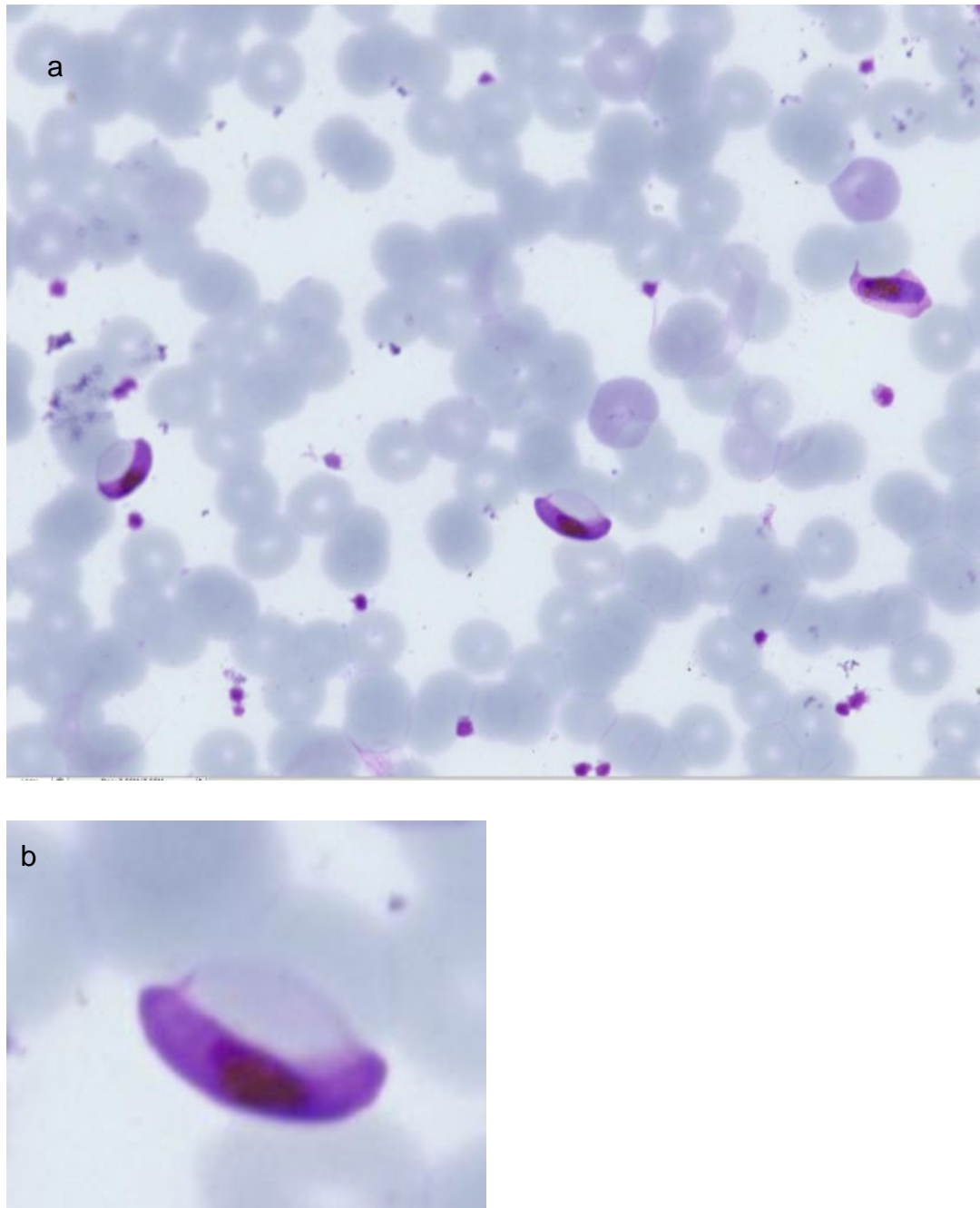


Figure 3.7 a and b shows the image taken *P. falciparum* gametocyte with the 5 MPx camera, b shows the isolation of single cell

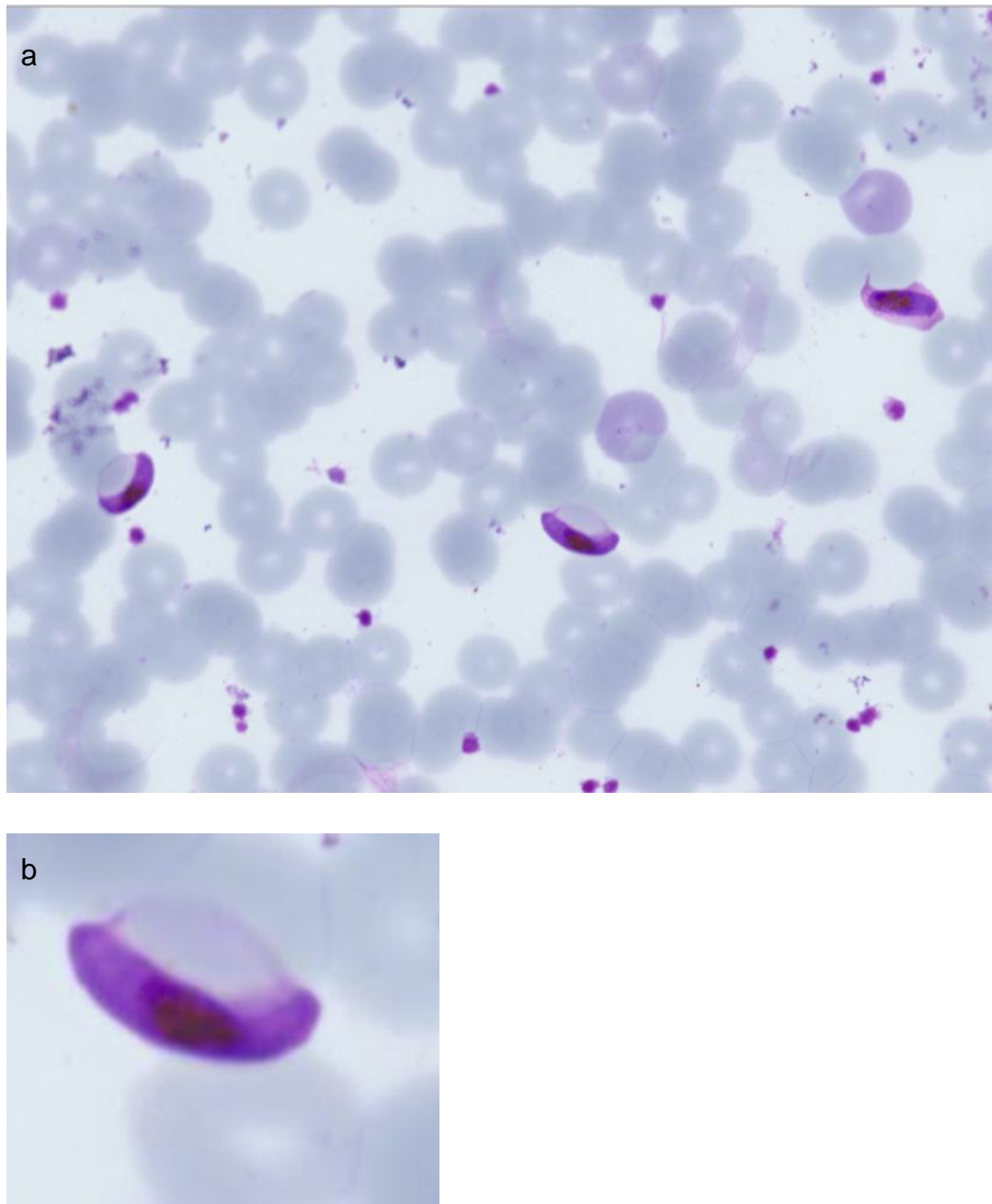
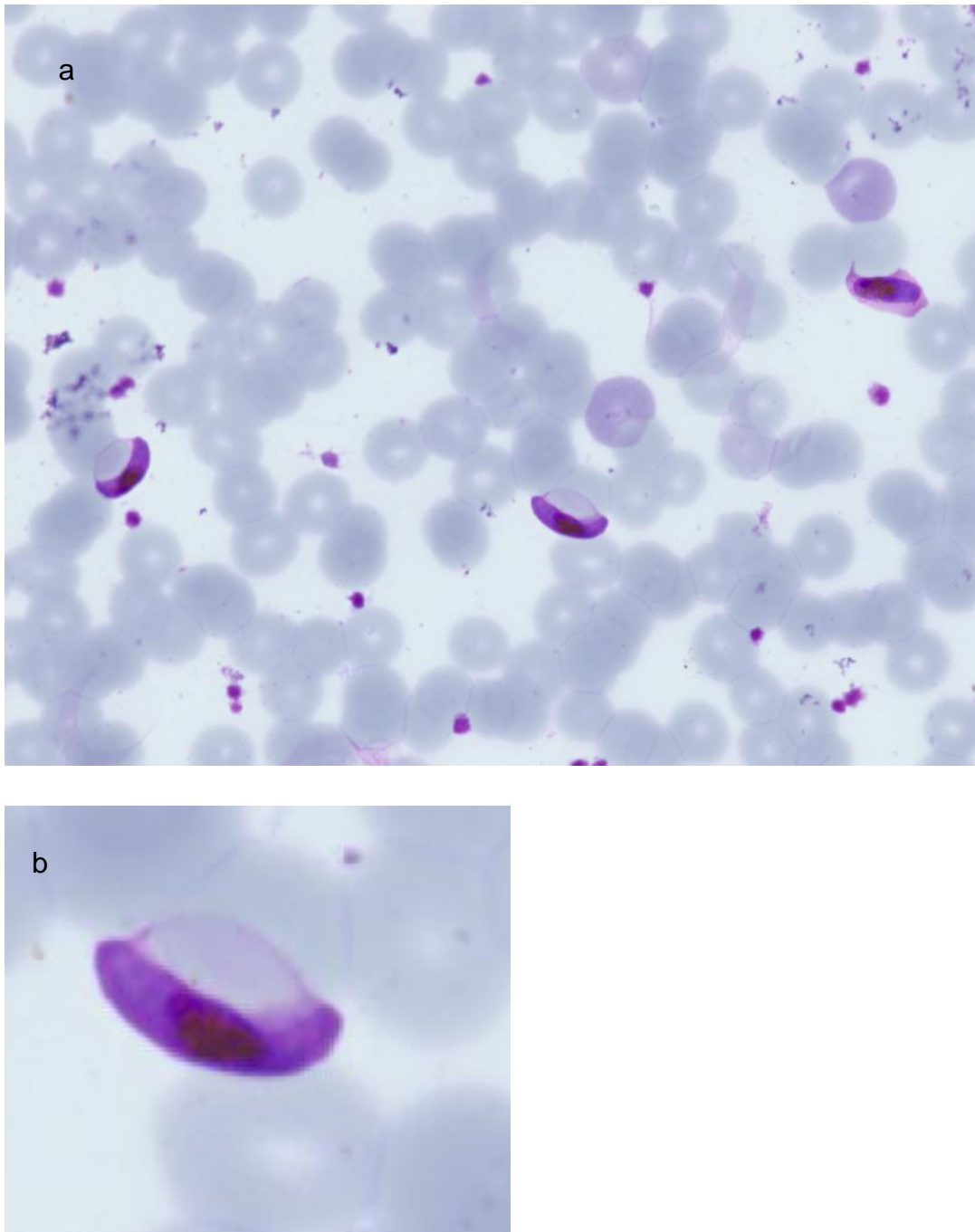


Figure 3.8 a and b shows the image taken *P. falciparum* gametocyte with the 12 MPx camera, b shows the isolation of single cell



Figures 3.6, 3.7 and 3.8 show the different camera resolution settings and the effect on the image when it is enlarged. These images have not been processed, however, the higher resolution image allows increased detail to be detected in the parasite and more effective display at higher magnification.

Focusing the image

Focusing the image can be difficult, especially if multiple parasites are present in different planes on the film. Figure 3.9 shows two parasites on the same image in two different planes. When parasites were present in different planes of view it can lead to a lack of detail being seen and can cause problems in species identification.

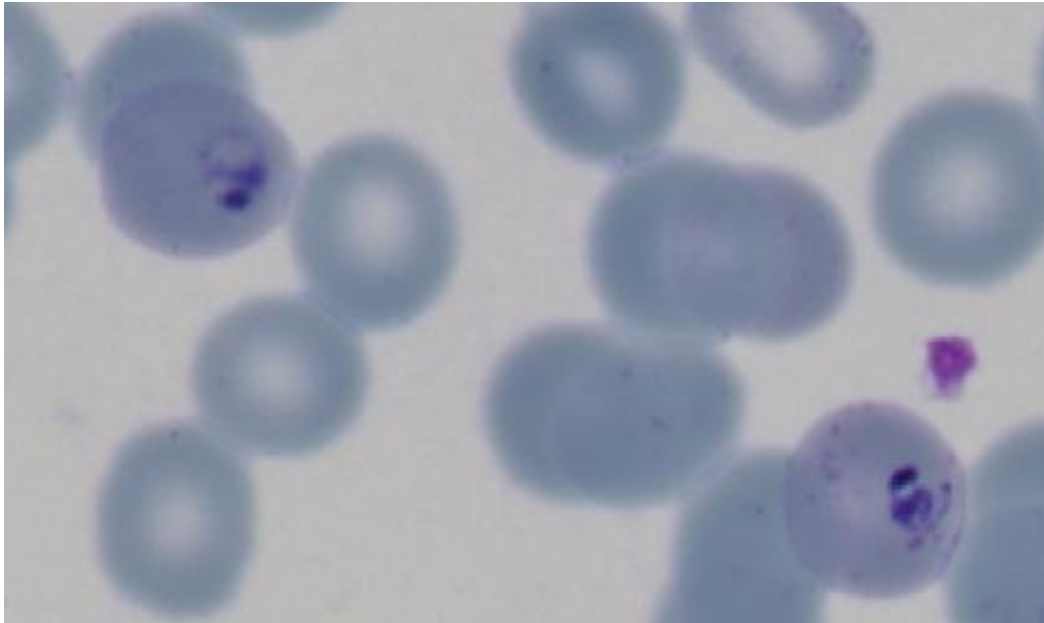


Figure 3.9: One parasite in and one out of focus due to a different focus plane

Automated vs. Manual stitching

Automated and manual stitching were tested to confirm what was the best method. Automated stitching also had problems due to focal plane selection and despite requiring less direct input at the time of imaging, solving problems that occur can take as long as stitching the image manually. Figure 3.10, gives examples of errors that can occur with automated stitching.

Stitching images

Zeiss microscope

Using the AxioVision software, the files can be directly stitched into a single image and then saved as a single file. The stitching function was accessible

immediately after completion of image capture in the “Acquisition” window. The stitching process is shown in figure 3.11. Once this stage had been completed the convert tile image function allowed for cropping of the image.

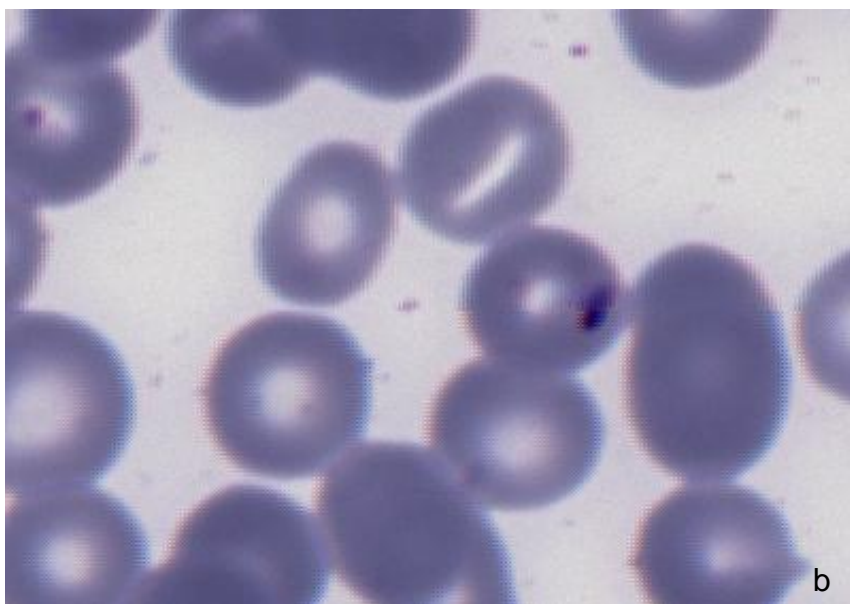
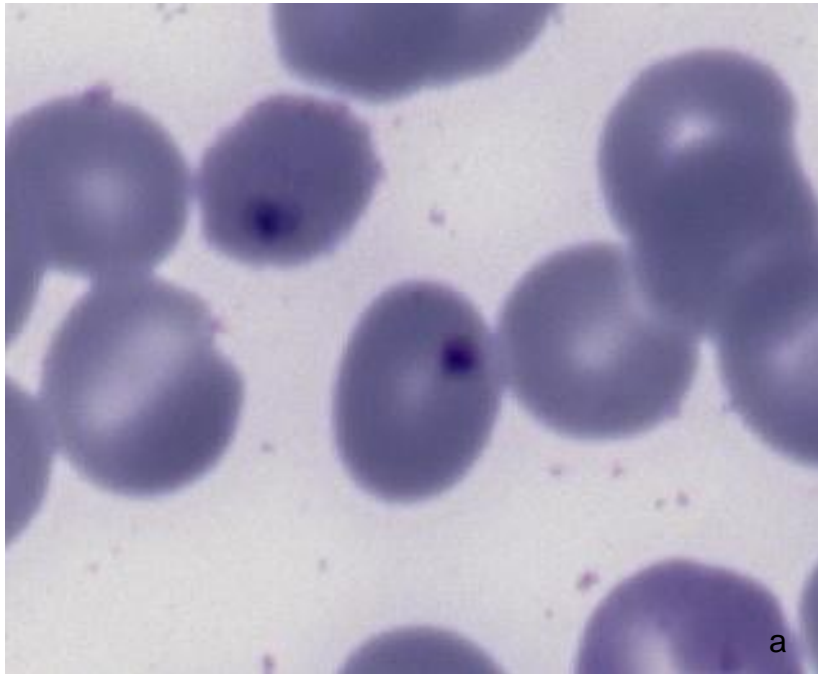


Figure 3.10 a and b, problems encountered with image generation using automated stitching. (a) shows an image with the parasites out of focus, (b) is not only out of focus but the stage has moved during capturing the image, leading to loss of image clarity.

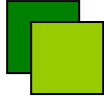


Figure 3.11: The stitching icon in AxioVision

Adobe Photoshop

The photomerge function (File> Photomerge>Automate) can be used to create mosaic images panoramic software in Photoshop CS3 (PS3). To allow images generated in the AxioVision software to be used in PS3, the files were saved as the TIFF format. One of the objectives for using PS3 was that individual captures images could be processed to enhance detail.

The auto arrangement function in PS3 was used, with the files either being selected from the folder in which they are saved or the window if they are already open. If there was enough overlap a perfect stitched image was produced, which was then cropped and layer flattened into a single image.

Poor images requiring replacement

Even after following a precise protocol some images like those shown in figure 3.10 would require replacement to ensure that the final large image was of the optimal quality required.

3.3.4 Conclusion

The 12 MPx camera was chosen to allow more detail to be seen at a higher magnification to simulate microscopy objective choice up to x100. Even though the size of the image was three times that of the 1.2 MPx Image, this was considered necessary to achieve the required image quality.

Due to difficulties in focusing the manual stitching method was chosen, with extra care being taken to ensure that the slide was flat on the stage, to try to prevent large differences in the focal plane.

The files were saved as TIFF format, to preserve image quality and to ensure compatibility with a number of systems. Alternative formats include .zvi a raw image format used by AxioVision, which was only accessible through

Axiovision, and therefore was not compatible with loading into the internet display software. JPEG images could have been chosen, however as the file would have to be saved a number of times before the image was uploaded onto the internet there were concerns over a loss of image quality with each save. For this reason JPEG images were not used, except at the final stage before image upload to the internet.

Adobe Photoshop was chosen to stitch the images, as the microscope software could be unreliable and often there were problems with the computer processing speed.

3.4. Image processing for online presentation

3.4.1 Introduction

To ensure that the images viewed over the internet were of the same quality as those seen down the microscope, a few image correction stages were introduced. Image enhancement was restricted to revealing detail using sharpening enhancement. To ensure images had a natural “microscope” appearance care was taken at the capture stage to avoid over enhancement of contrast, which can produce a bleached background.

3.4.2 Methods

Detail enhancement

Detail enhancement allowed features that may not be as defined as they are in the microscope image to be seen. The settings for enhancement were explored before using them in the images generated.

The software chosen for the detail enhancement was Digital Outback Photo (DOP)-Detail Extractor Version 2 (an Adobe Photoshop add-in). It was chosen after experimentation and comparison to other add-ins available at a similar cost.

Initially the images were converted into 16 bit images, to make sure that any changes made were on the highest quality image possible to prevent pixilation.

The settings for the DOP-Detail Extractor were explored for the images taken at different mega-pixel settings. There were six main settings, which could be adjusted in the add-in, they were

- **Detail Size:** Granularity of the detail to be enhanced
- **Boost:** Only needed for strong settings to amplify the effect
- **Extra Detail:** Increases detail enhanced
- **Protect:** Protect concentrates the contrast more to the midtones and helps to avoid problems at the edges of cells
- **Detail+:** Enhances the effect of Extra Detail
- **Clipping-:** Prevents extreme highlights and shadows.

http://www.outbackphoto.com/filters/dopf005_detail_extractor/DOP_DetailExtractor_V2.pdf

Different settings for these were also explored, varying these to achieve the best image.

Contrast masking

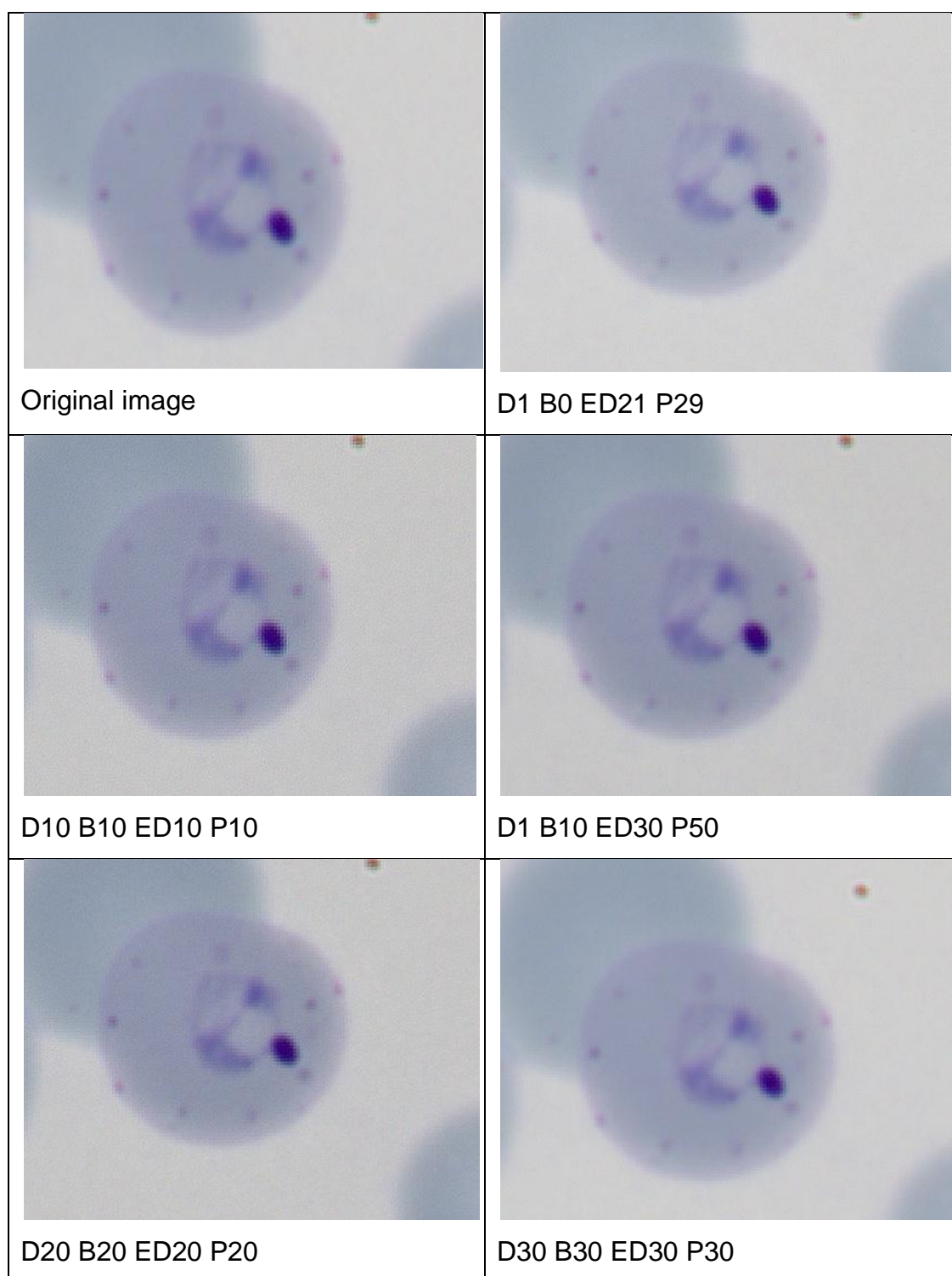
The contrast masking settings darken the image to even out the colour enabling the image to be more representative of the original. This allowed for better contrast between the background and the cells, allowing them to be seen more easily and reduced noise in the background.

3.4.3 Results and discussion

Detail enhancement

Different detail enhancement settings were examined for the 12 MPx images to generate the most realistic image. Single cell examples were shown in figure 3.12 to allow easy comparison.

Figure 3.12: Comparison of detail enhancement methods, Settings shown below image, D= detail size, B= boost, ED= extra detail, P= protect



Contrast mask

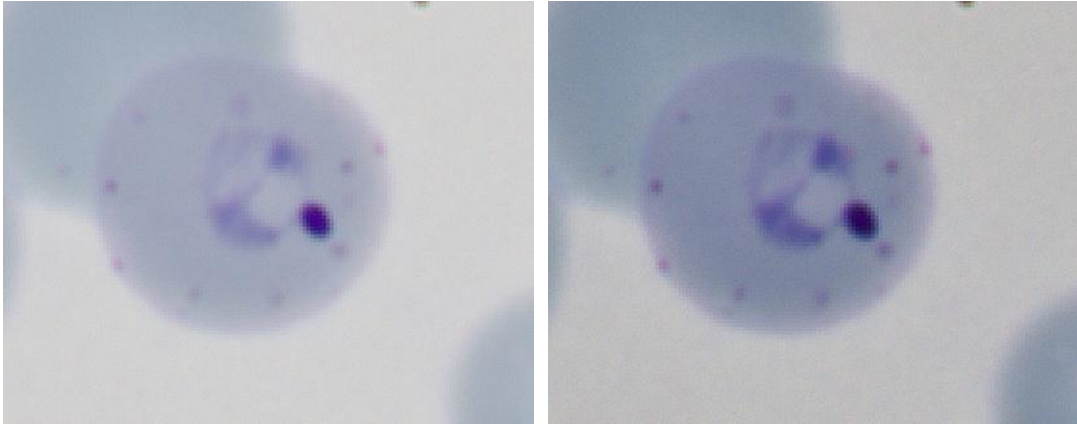


Figure 3.13: Comparison of the detail enhanced image before (left) and after contrast mask (right)

Figure 3.13 demonstrates the use of the contrast mask technique to darken the cell but also increases the detail present.

3.4.4 Conclusion

Looking at the images in figure 3.12 and 3.13 the final image processing procedures were chosen. The settings were as follows

The DOP detail extractor settings chosen were

- Detail size 1
- Boost 0
- Extra detail 21
- Protect 29
- Detail On
- Clipping On

Contrast mask settings

Convert image to 16 bit

- Duplicate current layer
- Desaturate duplicated layer
- Invert
- Set duplicated layer to 70% opacity
- Set duplicated layer to overlay
- Apply gaussian blur – 98.7 pixels
- Flatten image
- Convert image to 8 bit

These were deemed to give the best quality image in the processing time available. Thick and thin films were treated in the same way, to ensure consistency in processing.

3.5 Choosing images to be used for competency quality assessment and training

3.5.1 Introduction

Images were chosen to reflect the challenges that might be faced in a routine laboratory. These incorporated images of simple and challenging diagnoses. These include images from the four species that infect humans, *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* as well as negative samples.

To ensure that the images were of a comparable quality between the initial and final assessment preventing bias, the images were each assigned a classification for:

- Rank of the microscopic image difficulty
- Artefact rank
- Species
- Parasite density
- Thick or thin film
- Stage of life cycle present

3.5.2 Methods

Rank of the microscopic image difficulty

The rank of the image was determined by the difficulty of the diagnosis, the species present, the parasite density and the preparation of the specimen.

There are three values assigned to the rank of the image

1.
 - Easy to reach diagnosis
 - Few artefacts present on the specimen
 - Parasites are obvious and defined
 - Little stain deposit present
 - Well prepared blood film
 - Usually a high parasite density
2.
 - Moderate difficulty
 - Some artefacts are present
 - Parasites are less obvious
 - Stain deposit is present, but is usually generalised across the specimen
 - Some discrepancies in blood film preparation may be present
 - Parasite density will be lower than in rank 1
3.
 - Diagnosis difficult
 - Artefacts are present
 - Parasites are difficult to find

- Stain deposit may be present and may influence diagnosis
- Blood film may be poorly prepared
- Parasite density is usually very low (one to five cells present)

Artefact rank

The artefacts on the blood film ranged from stain deposit to the presence of high numbers of platelets, especially those that were present on top of the erythrocytes and could be deemed to influence the diagnosis made.

Artefacts were classified from 0 to 4.

0. No artefacts present
1. Few artefacts present, unlikely to influence diagnosis
2. Artefact present, some may be covering cells
3. Artefact present, numerous may prevent parasites being seen
4. Large numbers of artefacts present, may prevent parasites being seen, but also may be confused as parasites themselves.

Species

The number of cases of each species were chosen to reflect the routine laboratory workload. However, there were fewer negative cases, as the main aim of the exercise was to determine if they first identified that parasites were present and secondly determined the correct species. Negative cases were however used to determine whether false positive diagnosis was made and incorrect treatment for the patient, which could contribute to drug resistance.

P. falciparum cases were chosen to be the main species, as for the majority of the laboratories involved, this would be the only species seen. A limited number of *P. malariae* cases were available and therefore this was chosen as the species with the least cases presented.

Parasite density

The parasite density for all samples were split into three categories for analysis.

1. <5 cells present (<0.1%)- low parasite density
2. 6-49 cells present (0.1-1%)- mid parasite density
3. >50 cells present (>1%)- high parasite density

3.5.3 Results and discussion

In the initial assessment the 40 images chosen were all classified. To enable the final assessment to be comparable the images were chosen with the numbers of each being as close as possible.

Ranking of the microscopic image difficulty

Table 3.1 Rank of the microscopic image in the initial and final assessment

	1	2	3
Initial assessment	12	20	8
Final assessment	13	19	8

Artefacts rank

Table 3.2: Artefact rank of the cases in the initial and final assessment

	0	1	2	3	4
Initial assessment	7	8	8	8	9
Final assessment	5	8	10	13	4

Thick and thin films

There were seven thick films in the initial assessment and eight in the final assessment. In the initial assessment the seven cases were composed of four *P. falciparum*, one *P. vivax* and two negative samples. In the final

assessment there were eight thick film cases, three *P. falciparum*, two *P. vivax*, one negative, one mixed infection and one *P. malariae*.

Species

The number of images present for each species was controlled between the initial and final assessment (table 3.3).

Table 3.3: Number of cases from each species in the initial and final assessment

	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>	Mixed infection	Negative
Initial assessment	24	3	4	1	1	7
Final assessment	24	3	3	2	1	7

Each of these were then categorised to match those in the initial assessment

P. falciparum

The rank has been compared for the initial and final assessment in table 3.4.

Table 3.4: Number of *P. falciparum* cases at different ranks

	1	2	3
Initial assessment	11	9	4
Final assessment	11	9	4

The parasite density of the *P. falciparum* cases in the initial and final assessment is shown in table 3.5.

Table 3.5: Number of *P. falciparum* cases present at different parasite density ranks

	1	2	3
Initial assessment	12	6	6
Final assessment	12	6	6

The presence of artefacts in the initial and final assessment were taken into account. Table 3.6 shows the artefacts present in the initial and final assessment.

Table 3.6: Number of cases at each artefact rank in the initial and final assessment

	0	1	2	3	4
Initial assessment	5	6	4	4	5
Final assessment	4	6	5	6	3

P. vivax

The same process was carried out for the other *Plasmodium* species. As there were only a small number of cases of these the parasite density was not taken into account. All cases were present at mid to low parasite density.

The rank of the microscopic image was initially taken into account as in the initial assessment. Table 3.7 gives the comparison of cases in the initial and final assessment and the rank given to these cases.

Table 3.7: Rank of *P. vivax* cases in the initial and final assessment.

	1	2	3
Initial assessment	0	2	1
Final assessment	0	2	1

As there was only a small number of cases available controlling the artefacts present in the initial and final assessment was difficult. Table 3.8 shows the artefact ranking of the images present in the initial and final assessment.

Table 3.8: Artefact rank of *P. vivax* cases in the initial and final assessment.

	0	1	2	3	4
Initial assessment	1	0	1	1	0
Final assessment	0	0	1	2	0

The same procedure was used for the other species.

P. ovale

The rank for the initial and final assessment is compared in table 3.9.

Table 3.9: The rank of *P. ovale* cases in the initial and final assessment.

	1	2	3
Initial assessment		3	1
Final assessment		1	2

The artefacts present in the initial and final assessment for *P. ovale* cases is shown in table 3.10.

Table 3.10: Artefacts present in *P. ovale* cases in the initial and final assessment

	0	1	2	3	4
Initial assessment	1	1	2	0	0
Final assessment	1	2	0	0	0

P. malariae

In the initial assessment only one *P. malariae* case was chosen with a rank of two and three for artefacts. As two images were chosen for the final assessment on thick film was included. The images had a rank of two and three for artefacts.

Mixed infection

The mixed infection case was the same for the initial and final assessment, with an image from the thin film being used in the initial assessment, and from the thick film in the final assessment.

Overall, the images were chosen to be as close as possible to those used in the initial assessment to prevent bias occurring in the results.

3.6 The use of the online virtual microscope- SlideBox

3.6.1 Introduction

The virtual microscope system used for this project was used as it was already in use for haematology digital morphology by UK NEQAS(H) for their CPD scheme. The system was available without any additional costs to allow the provision of the images for training and educational purposes.

The Digital SlideBox system (figure 3.14) permits participants' interaction, allowing them to complete cases with individual questionnaires being attached. The system also allows annotations to be added to images, so that the individual could be given feedback on their performance.

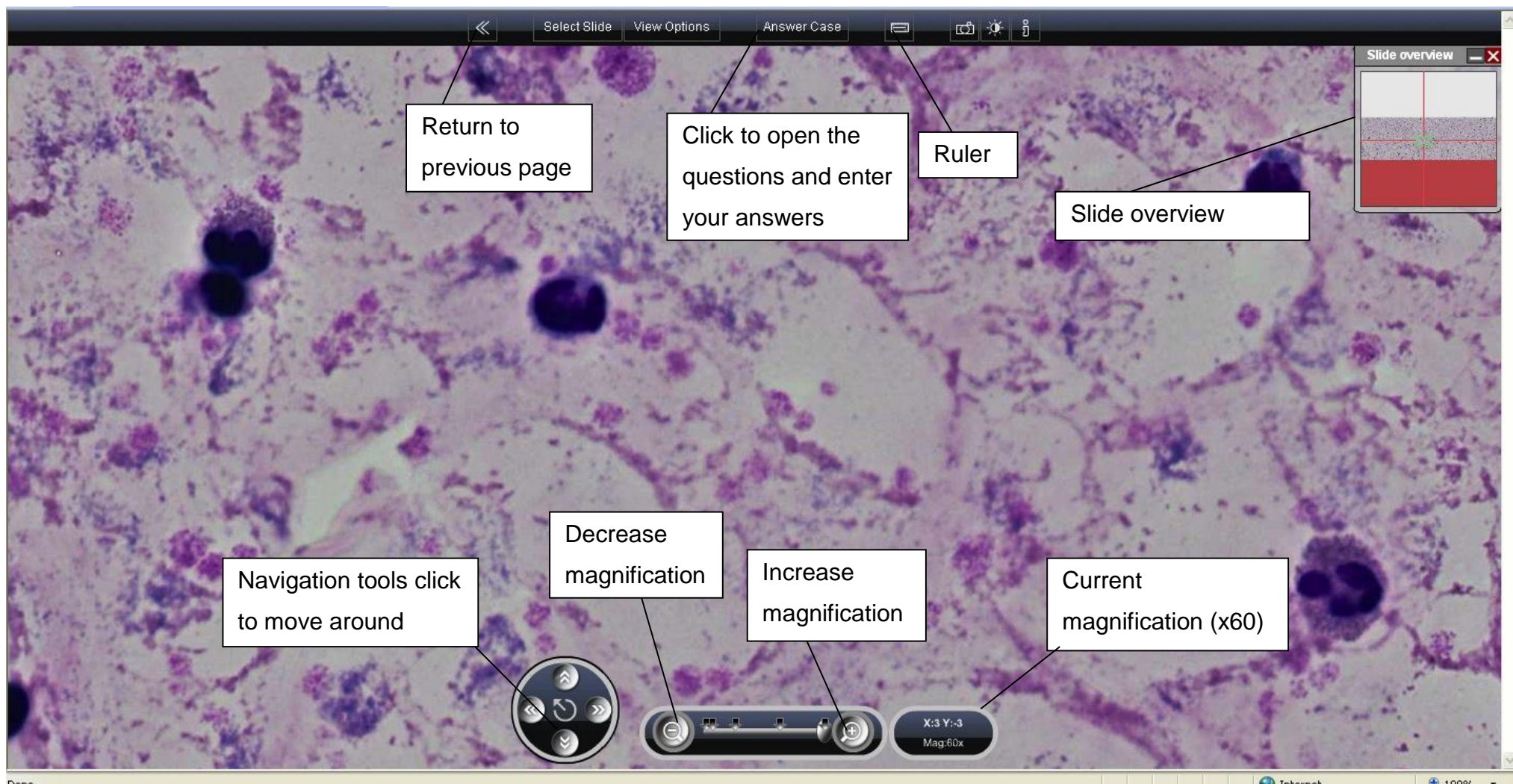


Figure 3.14: The SlidePath digital SlideBox environment

A number of stages are required throughout the online process to allow interaction with the images.

3.6.2 Methods

Converting file for upload to SlideBox

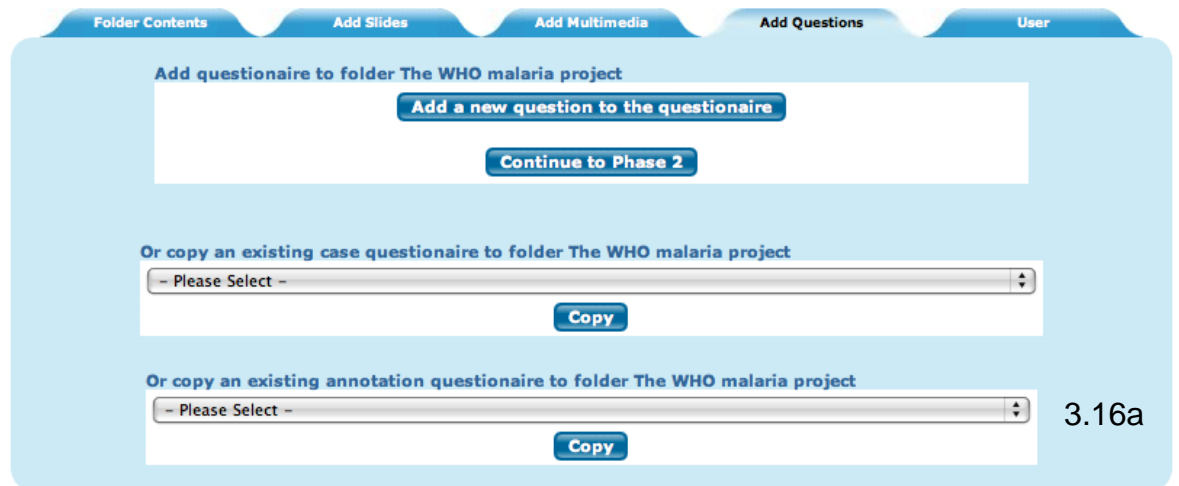
Initially the image has to be added to the system. SlidePath could not directly upload the TIFF images into their software environment as it was only set up to work with the SVS file format. The images were sent to the software company via file transfer protocol (FTP) over the Internet. Using Digital Slide Studio software the TIFF image was converted into an SVS file. This uses compression to make the image a tenth of its original size to allow quick access over the Internet and to apply the magnification to the image. There is a loss of image quality at this stage, which is why the sharpening procedures are used, to allow the image provided to be of the highest quality possible.

Testing the image uploaded into SlideBox

Once the image had been uploaded the software can then be set up to allow the case to be accessed directly on the virtual microscope administration pages (figure 3.15). The image can be selected from its location on the server using the add slide command.

Assigning questions to the image

Once an image was loaded, questions were added in the SlideBox administrator mode. There are the options to either add a questionnaire already present (figure 3.16a) or to add a new questionnaire. Adding a new questionnaire requires all the information to be entered manually (figure 3.16b)



The screenshot shows the 'Add Questions' tab of the SlideBox administrator interface. The main heading is 'Add questionnaire to folder The WHO malaria project'. Below this, there are three main options:

- Add a new question to the questionnaire**: This option is highlighted with a blue button. Below it is a 'Continue to Phase 2' button.
- Or copy an existing case questionnaire to folder The WHO malaria project**: This option is followed by a dropdown menu showing '- Please Select -' and a 'Copy' button.
- Or copy an existing annotation questionnaire to folder The WHO malaria project**: This option is followed by a dropdown menu showing '- Please Select -' and a 'Copy' button.

The interface has a blue header with tabs: 'Folder Contents', 'Add Slides', 'Add Multimedia', 'Add Questions' (selected), and 'User'.

3.16a

Folder ContentsAdd SlidesAdd MultimediaAdd QuestionsUser

Add questionnaire to folder The WHO malaria project

Enter a question to be displayedAre malaria parasites present in this image?

Is this question mandatory?☒

What type of score should be entered?

☐ Small Text (0 - 255 chars)
 ☐ Number
 ☒ Choice

Please choose the number of choices

2

Choice 1:

Choice 2:

Which best describes this Choice?

☐ Choose single
 ☐ Choose many

☐ Comment
 ☐ Measure

Add a new question to the questionnaire

Continue to Phase 2

Or copy an existing case questionnaire to folder The WHO malaria project

– Please Select –

Copy

Or copy an existing annotation questionnaire to folder The WHO malaria project

– Please Select –

Copy

Folder ContentsAdd SlidesAdd MultimediaAdd QuestionsUser

Add dependencies to questionnaire structure (optional)

Question Name

Type

Dependent Question Name

Dependent Question Answer

Are malaria parasites present in this image?

choice

– None –

Name and save questionnaire

Questionnaire Name:

Submit questionnaire

Figure 3.16a: Adding a questionnaire to SlideBox, add one formed or add new questionnaire shown in figure 3.16b and save it in figure 3.16c.

Chapter 3: Generation of microscopic images to be used for competency of diagnosis assessment

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Adding annotations

When an image is open, annotations can also be added. Annotations can either be in the form of a box, a circle or as an arrow. The area for the annotation to be added will first be chosen; once the annotation has been drawn information can then be added (figure 3.17). The feature can first be named, a description added and then placed into a group so that similar cells can be viewed together.

Preparing feedback

Feedback on the image is useful for the individual to indicate how they have performed. The narrative was written in the edit narrative box (figure 3.18), links can also be added either to annotations on the case, layers of similar features or to a separate website, in this case to the photo gallery website.

This provides all the stages involved in the generation of the stitched image. The processes involved in generated images for the gallery images will be described in the next section.

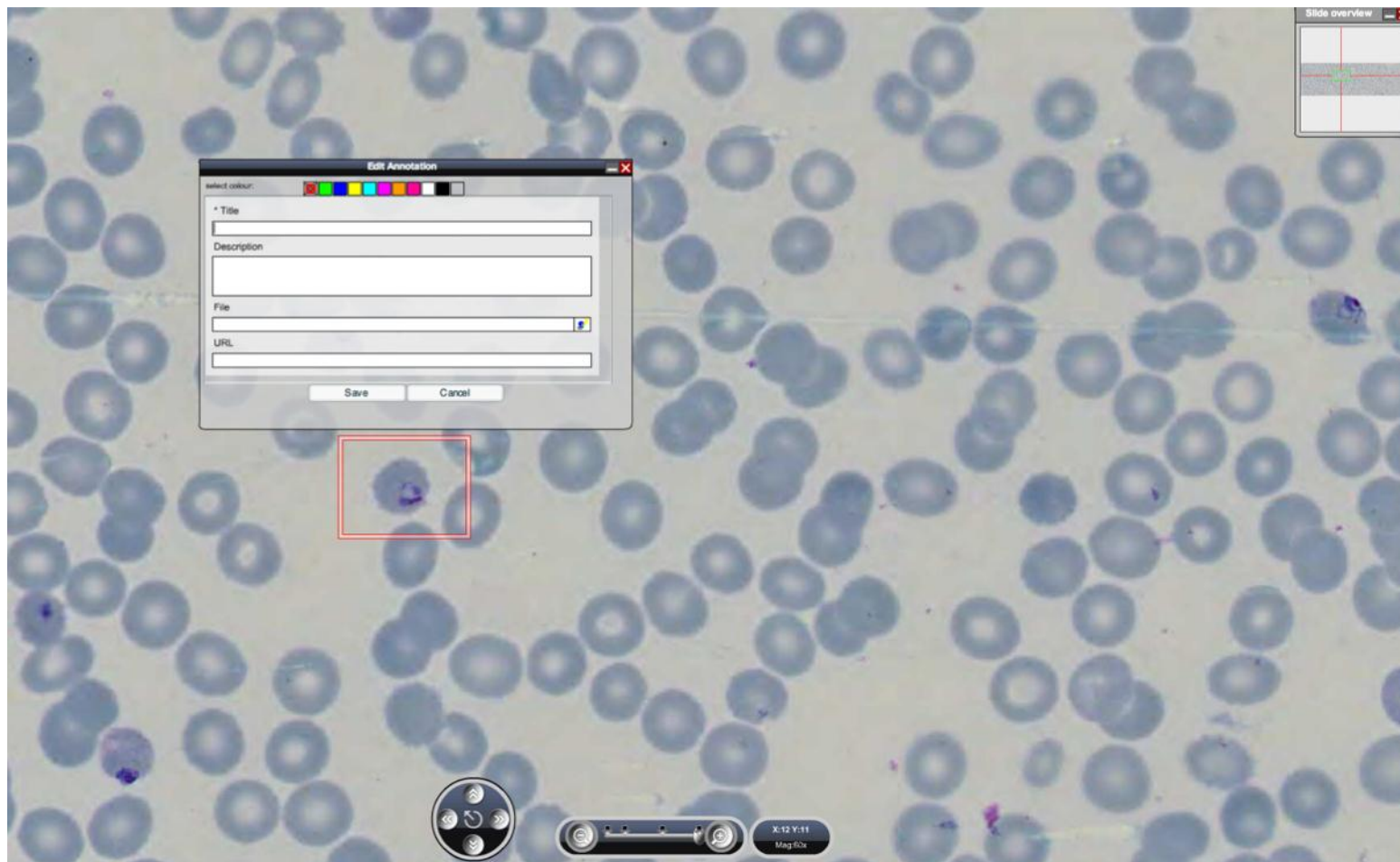


Figure 3.17: Adding an annotation to the stitched image, the black toolbar contains the square, circle or arrow tools to add the annotation

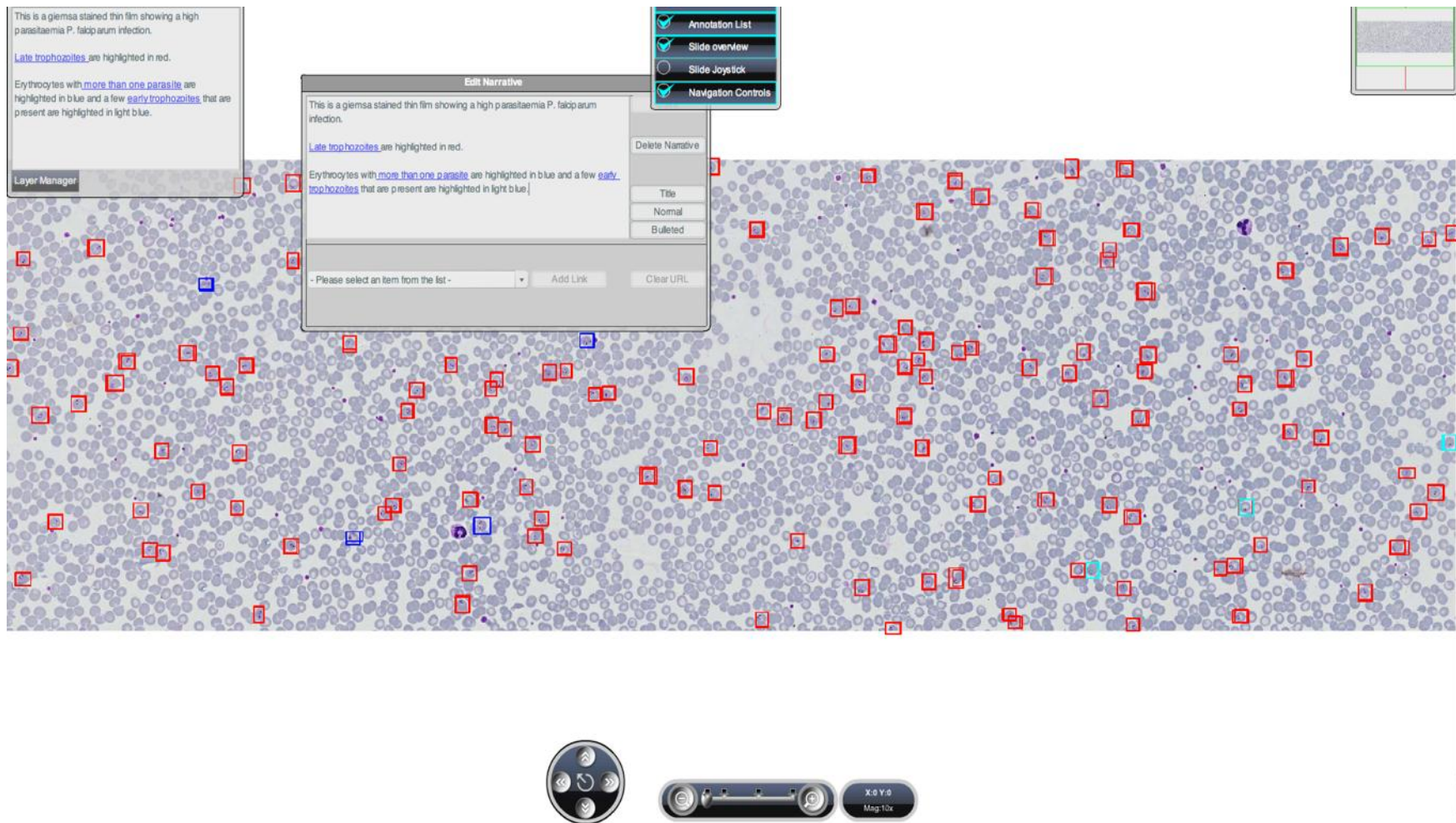


Figure 3.18: Editing the narrative to provide feedback

3.6.3 Discussion

There were difficulties with the upload of the 12 MPx stitched image as the image displayed incorrectly with the wrong magnification, making the image three times the normal size. The reason this occurred, was that the system based scale on a 1.2 MPx image and used the number of pixels to determine the size of the image and therefore the magnification. As at 12 MPx there were three times the number of pixels than at 1.2 MPx, the image appeared three times as large. The file therefore had to be converted to the size of a 1.2 MPx image in Adobe Photoshop before upload, as the sharpening and detail enhancement was done with the file at 12 MPx, this did not affect the image quality at the image magnification required. The upload converts the file into an SVS format, allowing the image to be viewed on the system.

When assigning questions to an image, difficulties were encountered with the wording of the questions. Any question that contained an apostrophe caused difficulties, the system was unable to identify these and therefore the questionnaire could not be completed. On removing the apostrophes from the questionnaire all of these features worked as would be expected.

Annotated images could only be of a certain size. The addition of too many annotations to an image caused the image to be slow to load and also caused annotations to be lost. For images with more than 200 annotations (i.e. more than 200 parasites present), some of these had to be removed, with only parasites with characteristic diagnostic features or EDTA changes being highlighted.

3.7 Overall conclusion

We have clearly demonstrated that it is possible to generate images of blood smears that can be used for quality assurance in order to improve the diagnosis and, thereby improve treatment, of haematological disease.

Generation of e-learning for the morphological diagnosis of malaria

4.1 Introduction

E-learning enables individual training without the trainer having to be present and is referred to by Nichols (2008) as “pedagogy empowered by digital technology”. However, Guri-Rosenblit (2005) explains that distance education and e-learning are not necessarily the same thing.

E-learning has been developed in many different areas of higher education, to provide initial learning materials (Laurillard, 2005) and to act as a vehicle of continuous professional development (Klein and Ware, 2003) and has been used in biomedical science education and training for some time (Ryan et al, 2000). Moreover, the use of a virtual microscope to enhance these activities, has been demonstrated to be of benefit to the learner when used in teaching and training in pathology (Sinn *et al.*, 2008).

E-learning offers many benefits over conventional teaching, but also has disadvantages associated with a lack of direct student supervision.

Benefits of e-learning (About E-Learning, 2007-2010; Littlejohn and Higgison, 2003)

- Allows working at students pace
- Access at anytime, anywhere
- Interactive environment
- Individual progress can be monitored
- Reduces transport costs
- Enables people living in remote areas and developing nations to receive education and material to which they would not normally have access
- Provides access to a range of resources which may not otherwise be available

- Encourages collaborative learning

Problems of e-learning

- Engagement of the learner into the training
- Lack of structure to simulate student effort
- Problems with internet connectivity
- Instructor not always available when required
- Isolation of students
- Collaboration when all inexperienced individual learners report false results
- Language used and understanding of training information

4.2 Pedagogy of e-learning

The arrival of the Internet has facilitated the enhancement of training in many different areas. This process is often called e-learning. The effectiveness of e-learning provision can depend greatly on the preferred learning styles of the individuals; therefore, learning styles should be taken into account during the design stage (Wang *et al*, 2006) of the programme. There are a number of learning style models that have been proposed, a brief description of each follows.

Kolb's model is based on experiential learning theory (Kolb, 1984). The Kolb theory is based on learning through experience, followed by observation and reflection on the experience. Abstract concepts can then be created, based upon the reflection, followed by testing the new concepts, which reinforces learning. Kolb determined that the ideal educational material provides all of these processes (Kolb, 1984). Individuals however, show strengths in specific areas, allowing specific learners to be identified

1. Converger- use active experience and abstract concepts to learn, making them good at practically applying ideas and solving problems using reasoning
2. Diverger- use concrete experience and reflective observation, making them imaginative, producing ideas and having the ability to see things from other perspectives
3. Assimilators- abstract conceptualisation and reflective observation, create theoretical models using inductive reasoning
4. Accommodators- concrete experience and active experimentation, engage by actions rather than reading and studying

Manochehr (2006) compared e-learning with traditional teaching for students of different learning styles. He showed that the learning style of the individual was significantly important for individuals undertaking e-learning but not for a laboratory based class. Assimilators and convergers performed better with the online training than with an instructor led course. Other studies have had difficulty proving the effectiveness of learning styles and have not reached consensus in their conclusions (Coffield *et al*, 2004).

Kolb's method has been adapted by **Honey and Mumford**, who initially renamed the phases in the cycle, to align them with problem solving and decision making processes (Mumford, 1995).

1. Having an experience
2. Reviewing the experience
3. Concluding from the experience
4. Planning the next steps

The styles were also renamed to Activist, Reflector, Theorist and Pragmatist. Two questionnaires were developed to help categorise learners, containing either 40 or 80 questions (Honey and Mumford, 2000). The self-assessment allowed the learning style to be used within industrial and commercial settings studied.

Gregorc also developed a learning style based around two perceptual qualities concrete and abstract and two ordering abilities sequential and random (Anon, 2008). A questionnaire was developed to categorise learners, based upon their responses to questions. There are four groups Concrete Sequential (CS), Abstract Random (AR), Abstract Sequential (AS) and Concrete Random (CR). This takes into account each individual's strengths and weaknesses, using the combination of learning methods.

The **Flemings Vark model** is one of the most common and widely used models (Leite, 2010). The model is an expansion on Neuro-linguistic programming models. Learners are classified as visual learners, auditory learners and kinaesthetic or tactile learners (Hawk, 2007).

Other models include the **Dunn and Dunn model** in which sociological and environmental factors are taken into account in addition to those in the other models combined (Hawk, 2007).

Alongside these basic learning models, specific models for e-learning programmes have also been developed to cover specific learning needs and also to include interactivity. Three models have been specifically developed for e-learning pedagogy (The University of Manchester, n.d.).

Mayes: The conceptualisation cycle. The cycle describes learning as a cyclical dynamic feedback process, having three components conceptualisation, construction and application, (Mayes and Fowler, 1999). Conceptualisation is focused on the transfer of knowledge from the teacher to the learner (Buzzetto-More, 2007). This involves the exposure to other people's ideas or concepts (The University of Manchester, n.d.). The later stages expand on the conceptualisation step, construction builds upon concepts. The construction is using the ideas they have been given and practically applying these to meaningful tasks (JISC, 2012). The application component, tests the conceptualisation component, using applied concepts. The goal is to test the understanding of abstract concepts, often developed during conversations and reflection with tutors and fellow learners, mainly through feedback on quizzes or tasks (Mayes and Fowler, 1999). The

learning is increased by constant feedback between the different stages in the process. To provide these components, three levels of learning are proposed, primary, secondary and tertiary courseware. The primary courseware presents the subject matter. The secondary courseware is the environment in which the matter is presented and the tools the learner uses. The tertiary courseware is material produced by previous learners, peer discussions and outputs from assessments (Mayes and Fowler, 1999).

Laurillard's conversational model (Laurillard, 1993) is based on the discussions between tutors and students. The model emphasises the use of communication within e-learning environments e.g. narratives (JISC, 2012). Interactions are designed to provide feedback, e.g. interactive feedback on outcomes of tasks and is used adaptively to revise the content for future groups. Discussion forums are also recommended, to allow the tutor to provide feedback and students can reflect on their achievements (University of Manchester, n.d.).

Salmon: 5 stage model and e-moderating, is designed for computer-mediated communication, the model is proposed as a five stage highly practical approach.

- *Stage 1: Access and motivation*, providing quick and easy access to the virtual learning environment
- *Stage 2: Online socialisation*, becoming comfortable with the online environment
- *Stage 3: Information Exchange*, Interactivity with virtual learning environment and e.g. web links, databases case studies and fellow learners.
- *Stage 4: Knowledge Construction*, building online communities focusing on learning.
- *Stage 5: Development*, taking responsibility for their own learning and becoming more confident and critical thinkers. (Salmon, 2003)

Mastery for learning was initially proposed by Bloom (1968), in 1971 this was modified to mastery learning (Bloom, 1971). Mastery learning proposes

that all students can learn to any level given enough time. The approach is to allow each student to complete a section and making sure they achieve a predetermined level of achievement before moving onto the next stage (Block and Burns, 1976). The level of achievement needs to be predetermined to ensure the level achieved at this stage is high enough to allow individual's to progress.

Mastery has been used in a number of studies, Barsuk (2010) reported on the use on mastery learning in the training of venous catheter insertion. Simulation-based mastery learning requires students to meet a required standard before progression to carrying out testing on patients. The results from the mastery training showed that skills acquired were substantially retained.

Leonard and Gerace (2010) report the use of mastery learning in the teaching of Physics at the University of North Carolina. Due to poor performance in one of the modules a prerequisite course was developed to improve understanding. In order to progress onto the module students were required to reach the desired level of mastery.

A number of studies in medical education have assessed the learning styles of the students, using a number of different methods. Zeraati *et al*, (2008) used the VARK questionnaire to assess the learning styles of their students. The majority of these students were auditory learners.

Lujah and DiCarlo (2006) also carried out the VARK assessment on a group of first year students. They discovered the majority of the students used multiple learning styles (63.8%), with the highest individual category being kinaesthetic at 18.1%. Similar results were seen by Johnson (2009) with 52.4% of their first year students using multiple modes of learning. However, a group of students were investigated who had been admitted to the university from targeted groups, these individuals were split between multimodal (28.1%) and kinaesthetic (28.1%). There were no auditory learners amongst the standard admission students, however in the targeted group there were 12.5%.

Engels and de Gara (2010) used Kolb's method to assess medical students in comparison with surgeons. The medical students were mainly assimilators, however qualified surgeons were mainly convergers. This indicated a different teaching mechanism was required for students and surgeons. In another study with medical students, Danish and Awan (2008) found that the majority of their students (54.6%) were accommodators.

However, Rohrer and Pashler (2012) argue that learning styles have no effect on medical education, as a review of the data shows no significant difference in results achieved. Due to the cost of assessing the student and re-writing some courses, they argue that the change is not cost effective for limited, if any, benefit for the students. They say "educators should instead focus on developing the most effective and coherent ways to present particular bodies of content, which often involve combining different forms of instruction". A principle that can be applied to online delivery of cell morphology using the virtual microscope.

Most studies on the use of virtual microscopy have been in histology teaching. Harris *et al*, (2001) compared a virtual microscopy laboratory and the regular microscope laboratory for teaching histology. Harris concluded that the virtual microscope is a viable addition to, if not a replacement for microscopes and glass slides. The students also preferred the use of the virtual microscope to the standard microscope. Jonas-Dwyer *et al* (2011) investigated the use of learning styles with virtual microscopy teaching. They used the ASSIST inventory to assess the learning styles of the individuals, both at the beginning and end of the teaching period. The ASSIST inventory categorises individuals as deep, strategic or surface apathetic learners. The study showed an increase in the number of deep learners as the course progressed, indicating increased involvement with the microscope over time. This change in approach was not observed in students solely using the laboratory based microscope and traditional teaching.

There are a number of training schemes for education in the diagnosis of malaria, histology and haematology, but the authors have not indicated

whether learning styles were taken into account during the development of these training programmes. The WHO malaria microscopy teachers guides (2010b), describe how to provide a training programme for malaria diagnosis, but no consideration of learning styles seems to have been taken into account.

Goubran and Vinjamury (2007) designed a tool for selective directed learning in histology, using an atlas-based approach. The atlas was shown to significantly improve the results of students who used the system, compared to those with no access and was highly popular with students.

Other training guides developed for malaria diagnosis include World Health Organization (2010b), World Health Organization (1999), and Shoklo malaria research institute (2002), none of these used learning styles in the design of the training.

The ideal training programme for malaria diagnosis should be designed around the mastery learning approach. With an unlimited time frame students would be able to work at their own pace to achieve high levels of competency on the recognition of each parasite stage and species before moving onto the next.

4.3 Intervention package content

To enable the delivery of training materials a number of potential issues were considered.

4.3.1 Target audience

In generating an e-learning programme the target audience should always be the initial consideration (Ismail, 2001). The audience was laboratory scientists, from around the world, possibly with English not being the first language.

Participants had different experience levels, which were determined using the questionnaire. The data provided was then used to categorise the participants' results in the initial and final assessment.

Learning styles of the individuals were also considered, the training was designed to cover all possible learning styles deliverable via e-learning. Visual and kinaesthetic approaches were covered, however auditory was not, as the computers in use may not have had audio capability.

4.3.2 Assessment of material that was already available on-line

A variety of websites are available for the diagnosis of malaria, some giving information about the malaria parasite in general, others giving diagnostic information. The WHO offers a recently updated training guide to be used in the diagnosis of malaria (World Health Organization, 2010b). The guide gives basic information about malaria and laboratory techniques involved in its diagnosis. A few examples of the parasite's appearance at different stages of development are given, with one image of each stage of development of the protists. The WHO generates bench aids (World Health Organization, 1999) to go alongside this information. These aids provide limited examples of parasite species and stages, but do use photographic examples of parasite appearance. Most provide single examples of cells and provide no interactive challenge.

The Shoklo Malaria Research Unit (2002) in Thailand have also generated an in house training guide, based on a modification of the original WHO guide (World Health Organization, 1991). There is more extensive information about blood morphology in general and further malaria examples. Methodology is also considered in this guide, with criteria for assessing the quality of staining given. Images provided are of poor quality, with the colour of the images, in some cases, giving misleading representations.

There are also a number of different websites that provide similar information to these training guides. The Center for Disease and Control website (Centers for Disease Control and Prevention, 2008) lists information about all

tests carried out in diagnosis, and also provides links to galleries of images of individual parasite infected blood cells, the equivalent to the bench aids produced by the WHO.

The Royal Perth Hospital, Australia (Royal Perth Hospital, 2003) website gives information on the laboratory diagnosis of malaria and offers an engaging teach and test section. This website asks users to view a patient case and determine, from the images given, whether malaria is present and to identify the species.

The UK NEQAS parasitology scheme has a website dedicated to the diagnosis of malaria (United Kingdom Quality Assessment Scheme for Parasitology, 2006). This website gives details of the methods required for the accurate diagnosis of malaria and the effects of storage of whole blood in EDTA on the morphology of the parasites.

Material available to laboratory workers to aid the diagnosis of malaria was discussed with recognised experts who identified some problem areas in the diagnosis of malaria. Species identification was highlighted as a major issue, along with the quantification of the parasite density and microscopic slide preparation (Williams, 2009). These issues were all addressed within the project, by including images and examples to compliment the written text.

Upon assessment of the currently available learning/ training material the following topics were identified as being essential to the proposed training schedule:

- Laboratory methods
 - Problems associated with incorrect preparation of the blood smear
 - How to avoid problems in preparation of microscopic materials
- Background information about malaria
- Assessing the presence of parasites

- Appearance of parasite stages
 - Information of life cycle stages
 - Multiple images of each stage
- Species comparison
 - Details of differences between species
 - Images showing variation in morphology with the same species
- Quantitation of parasite density
- Thick films versus thin films

4.4 Intervention package structure

An integrated training package capable of being offered to laboratory workers currently undertaking microscopical analysis of malaria parasites. This package would allow participants to study at their own pace, to their own knowledge level and learning style.

4.4.1 Participant experience and knowledge

The content of the training was designed to build on the current knowledge the participants already had and allow them to further develop their skills. The format of the training would, therefore, take this into consideration, facilitating individuals with more experience to benefit from the training given.

To this end, training material was initially generated for three different experience levels

- Basic - newly qualified staff or those with less than 1 years experience
- Intermediate laboratory staff with considerable experience working in the laboratory for 2 – 5 years
- Advanced- senior staff with more than 6 years experience

However, the file structure this created was complicated, and made navigation difficult, especially at this stage when Microsoft Word documents were being used. To solve this, one document was developed for each topic with increasing complexity being delivered as the participant progressed through the single document. To encourage engagement, a quiz would be placed at the bottom of each page. The feedback associated with this process would allow the participants to revisit any areas of the learning material in which they had difficulty in understanding. Therefore, the participants did not have to categorise their experience and therefore their training was optimised.

4.4.2 Participant guidance

To enable the participants to study the programme, guidance was given to ensure that they understood the objectives of the study and what they were expected to achieve (learning objectives). The guidance provided help and information at every stage of the learning material.

Although the training programme contains background information about malaria, the main purpose was to improve the detection and identification of parasites. Participants were, therefore, guided to study this area first, which in addition contained substantial information about diagnosis.

The participants were provided with a “how to study” document along with the information provided on the initial access page.

Participants also had email contact with the author at all times to allow any other questions to be answered. The quizzes were provided with a submission button, to allow them to submit the results of any quizzes if they wanted any further feedback.

4.4.3 Structure

The structure of the training aimed to be sequential, to help the participants progress from one stage to the next. There was also the facility to link

between the different stages to enable participants to refer back to information on any points that had not fully understood.

Each section of the training was designed to be used independently. The format of which was initially developed as giving the background information, some images of explanation and a quiz to confirm understanding. As there were participants with different levels of experience and training, the information for each of these individuals needed to be focused for their particular group.

The structure of the programme was designed to provide independent study and engagement. A side bar was created to provide links to relevant pages, as well as from links within the text. Links were initially made between word documents and then converted into HTML links.

4.5 Format of delivery

4.5.1 Introduction

The speed of the Internet connection available varies around the world. The training would need to take the speed into account, to ensure that all participants could easily access the training. Alternative mechanisms of delivery were investigated below.

4.5.2 Methods

Developing the delivery mechanism

To deliver the training programme, the initial delivery mechanism was a “Google site”. To achieve this pages were initially generated in Microsoft word, which could be uploaded directly onto the site. The generation of the site is shown in figure 4.1, where the template is chosen, before naming the site.

Figure 4.1: Creating a web page as a Google site

Once the site was generated pages can be added to it, adding a page is shown in figure 4.2. This window is figure 4.2 is accessed by clicking at add page icon in the opening window.

Figure 4.2: Adding pages to the Google site

Once a page was created content can be added to it. Figure 4.3 shows the list of content that can be added.

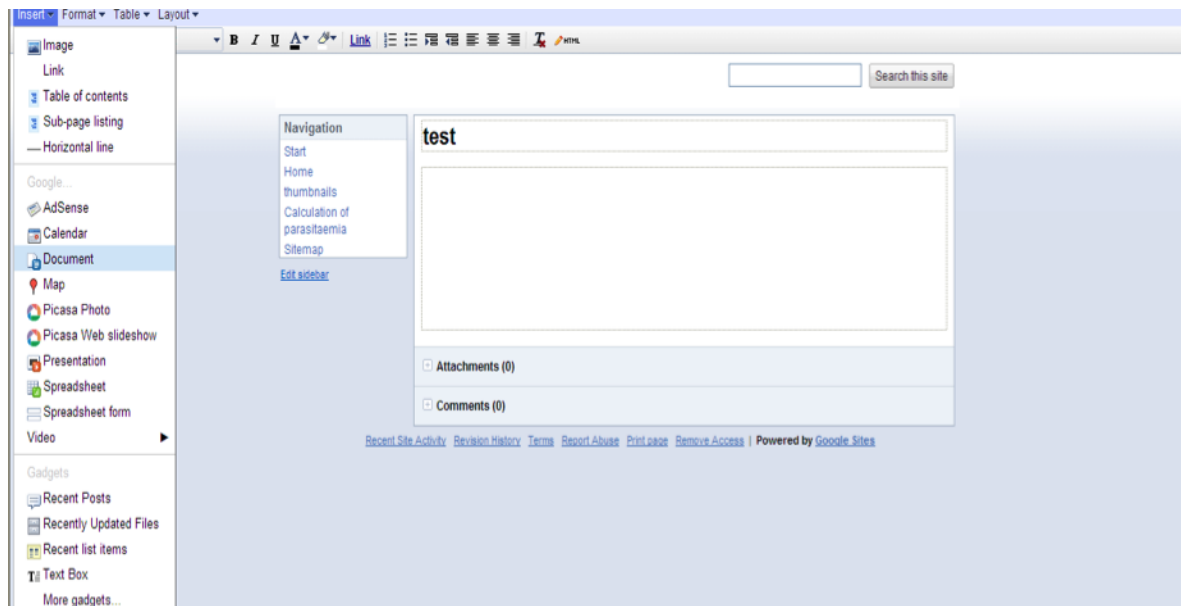


Figure 4.3: Adding content to a Google page- choose from calender, document, map, photos, slideshow, presentation, spreadsheet, spreadsheet form or video.

Before documents from Microsoft Word can be added they must be converted into a “Google document” (figure 4.4).

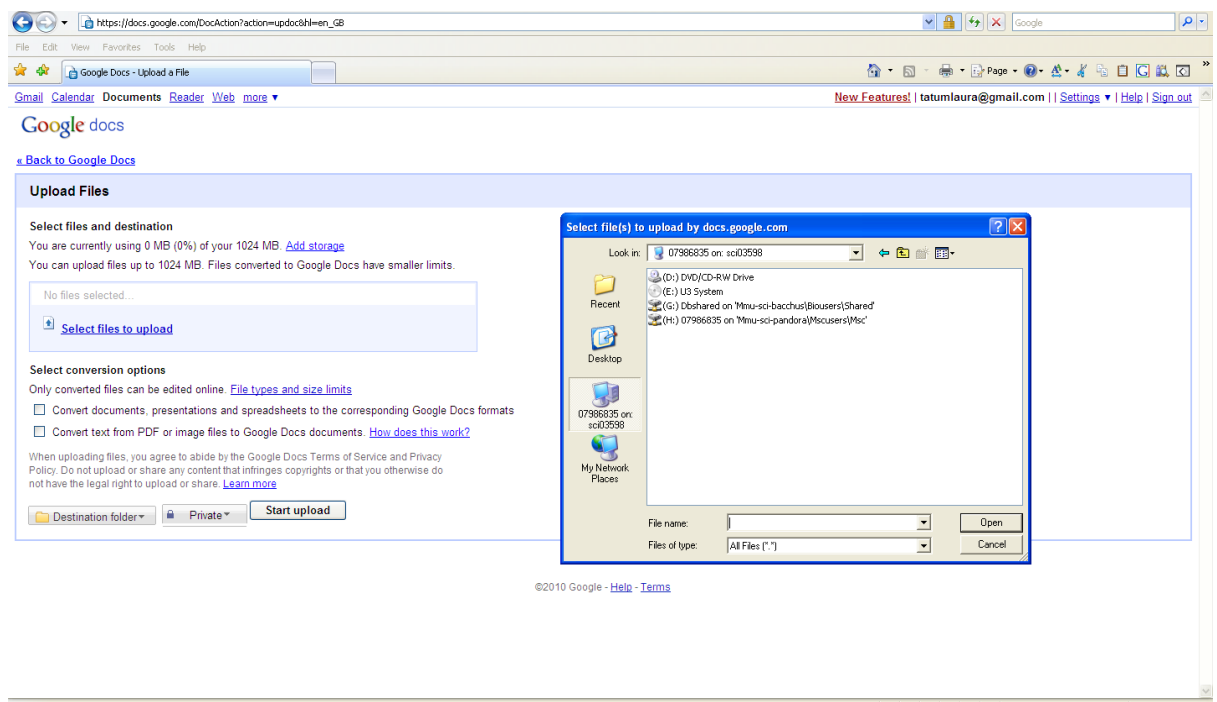


Figure 4.4: Uploading a document as a Google document

Once a document was uploaded it could then be placed into a web page as in figure 4.3. The size of the file sometimes needed to be changed to allow it to fit into the frame (figure 4.5).

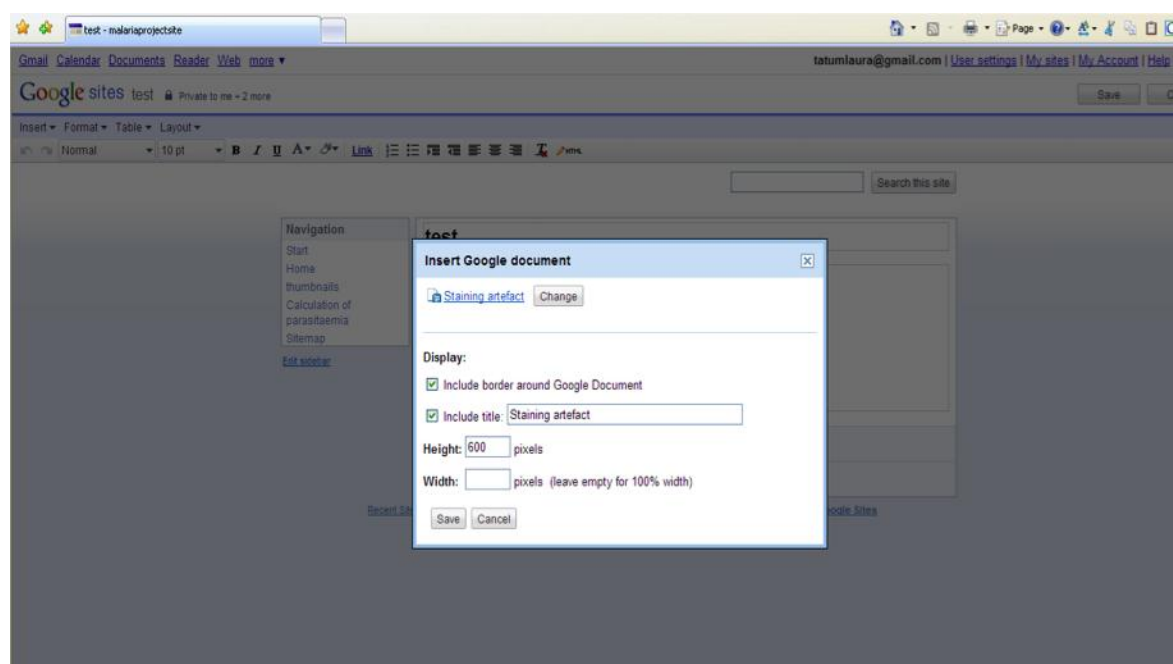


Figure 4.5: Inserting a Google document, setting the size of the screen

Once the file was inserted the content may only be edited in the Google document and not on the web page itself.

Inserting a gallery into the site was also carried out using the “Google document” generator. Initially a table was created, into which the images were inserted as shown in figure 4.6, (the size of the file was chosen to be constant with a width of 100 pixels).

The link to the full size image was created by making a separate Google document with thumbnail size images and linking to the original file as is shown in figure 4.6. The link from this file was then added as a hyperlink with the text “Click for full image” (figure 4.7).

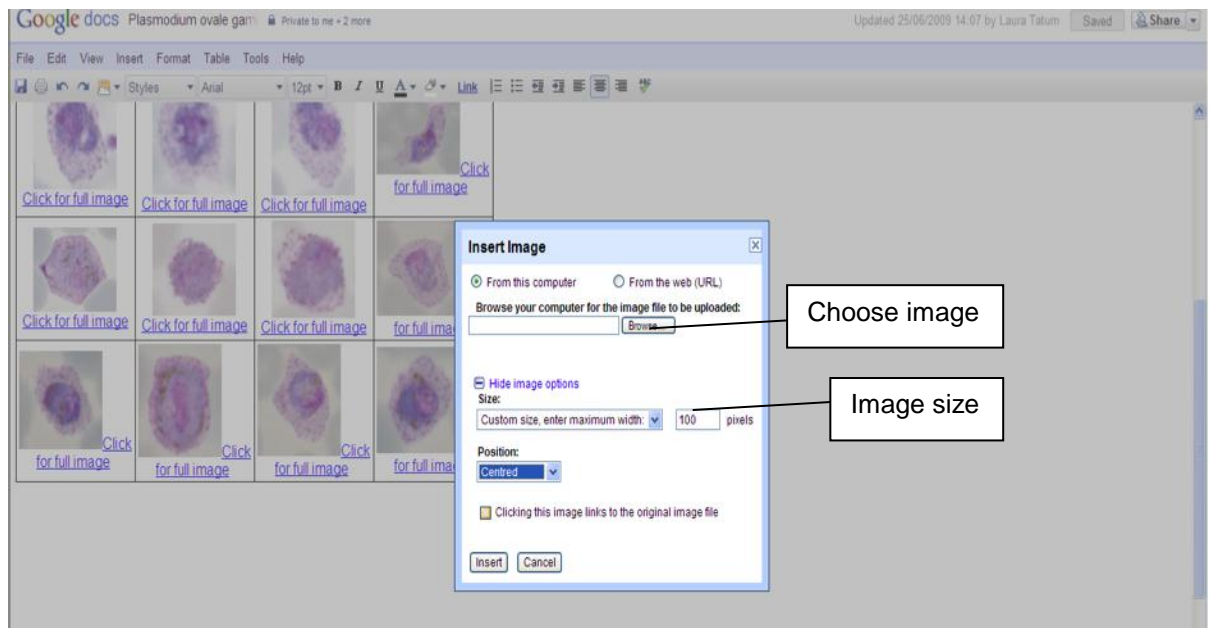


Figure 4.6: Inserting an image into a table for gallery format

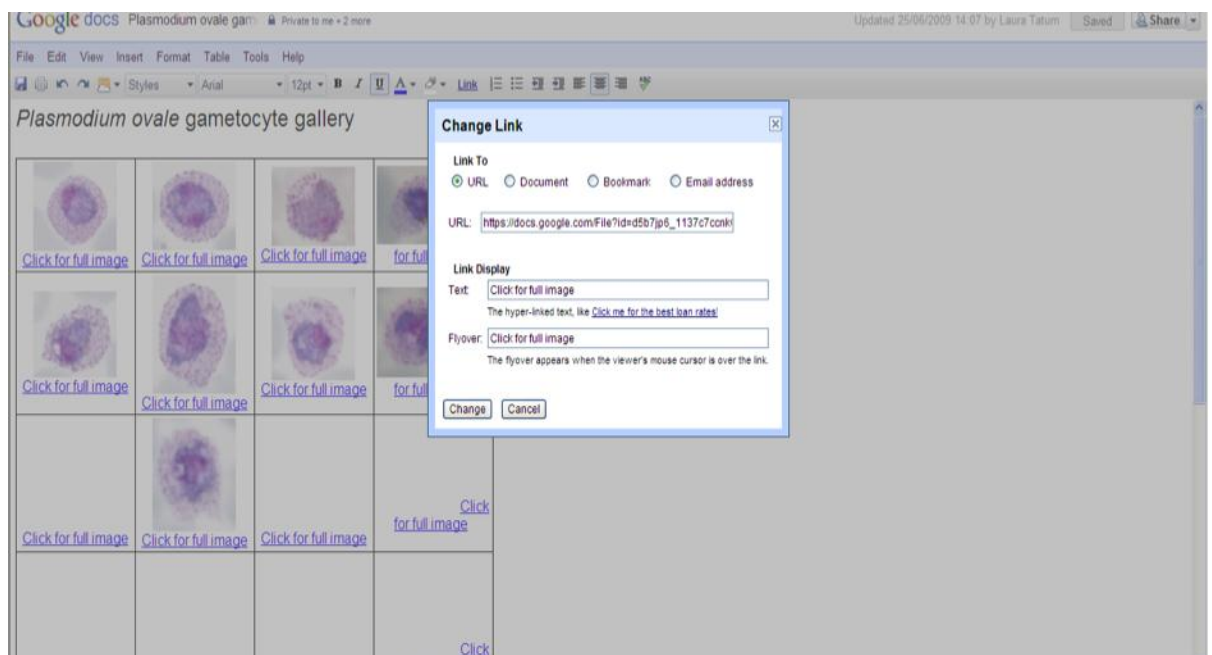


Figure 4.7: Adding links to the full size image

4.5.3 Results

Once the Google site was generated, problems with distribution to participants were found and the system could not be delivered on a USB stick or via CD-ROM. Therefore to enable the participants to access the site from a single location, an alternative delivery mechanism was sought. To allow this site to be run offline and then placed onto a different site, every

page and individual image in the site had to be saved as a complete HTML file (figure 4.8).

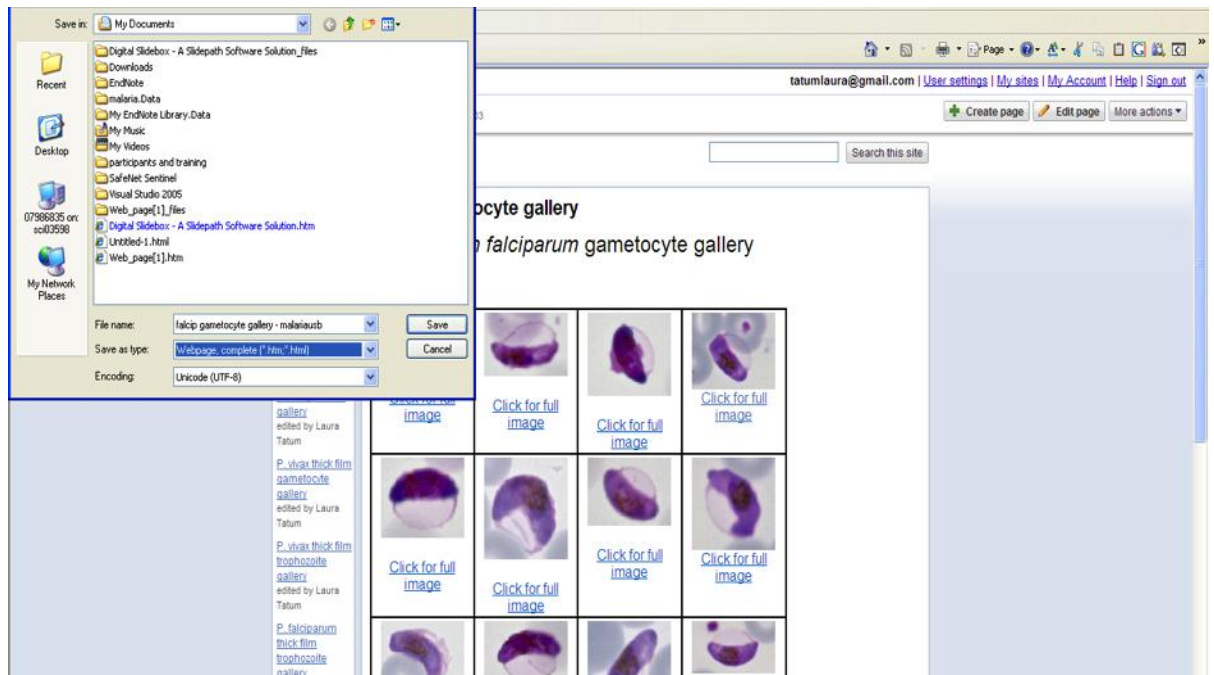


Figure 4.8: Saving Google pages to allow editing away from the Internet

The site was then edited using Adobe Dreamweaver 8. Links were changed to the file in which they were saved, all links were absolute (if the file was saved in another location the link would also change) (figure 4.9).

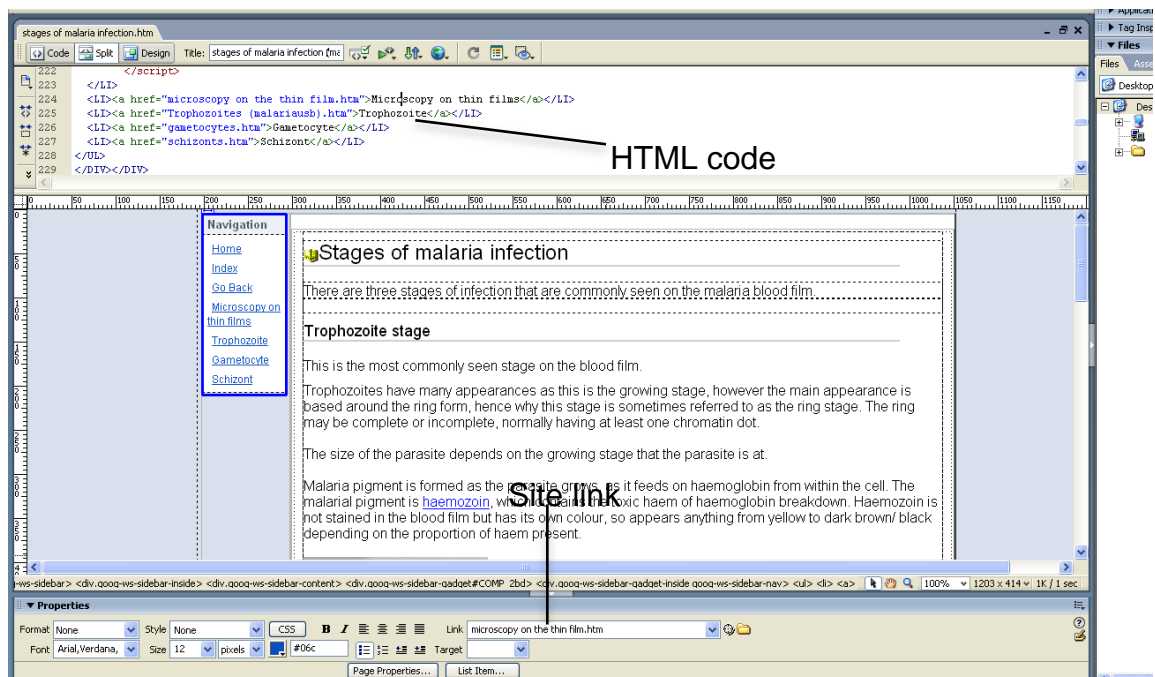


Figure 4.9: Adobe Dreamweaver to edit links in the web page

The programme was then operational on a USB stick or CD-ROM, however it still needed a location on the Internet where the file could be located easily by participants.

SlidePath hosted the training site on a server held at their Head Office allowing the participants to have access to the training through the site that they accessed the virtual microscope. The files were transferred onto the server using FTP. The link to the file in SlideBox was created by adding a multimedia link to the URL of the training programme site (figure 4.10).

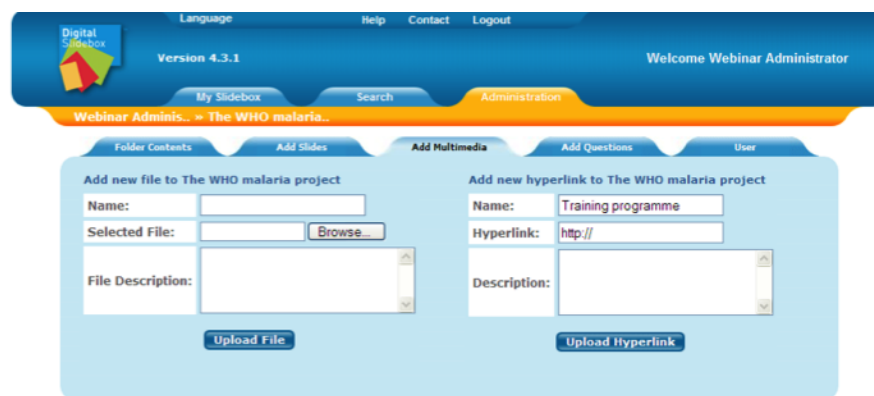


Figure 4.10: Adding a multimedia page onto the Slidepath site

4.5.4 Discussion

The participants were able to access the site from the one location, logging on to the SlideBox site and then being linked into the training programme. This would allow participant access to be monitored to check that they were participating in the project. The project was also available on a USB stick if required, so it could be sent directly to the participants.

4.6 Developing interactive feedback

4.6.1 Introduction

As the training programme was to be delivered via distance learning the only interaction the participants would have was using the assessment and

training material provided on one central website. Interaction was provided with items such as quizzes and immediate feedback. The quizzes needed to emphasise the information given on each page to check understanding and highlight areas that needed to be revisited. The quizzes tested on cellular recognition as well as background information given on the pages.

4.6.2 Methods

The provision of these quizzes was investigated, the type of quiz had to be chosen, as well as how feedback was going to be given. The mechanism of delivery for the quizzes had to be determined. As the quizzes required feedback the initial method used HTML files with hyperlinks to either the correct answer or a feedback on the response given. This method however did not allow the participants to judge how they had done on the test.

This led to the exploration of using Flash based quizzes. Initially using Abode Flash Professional CS3 a quiz template (figure 4.11) was used to generate the basic layout of the quiz pages.

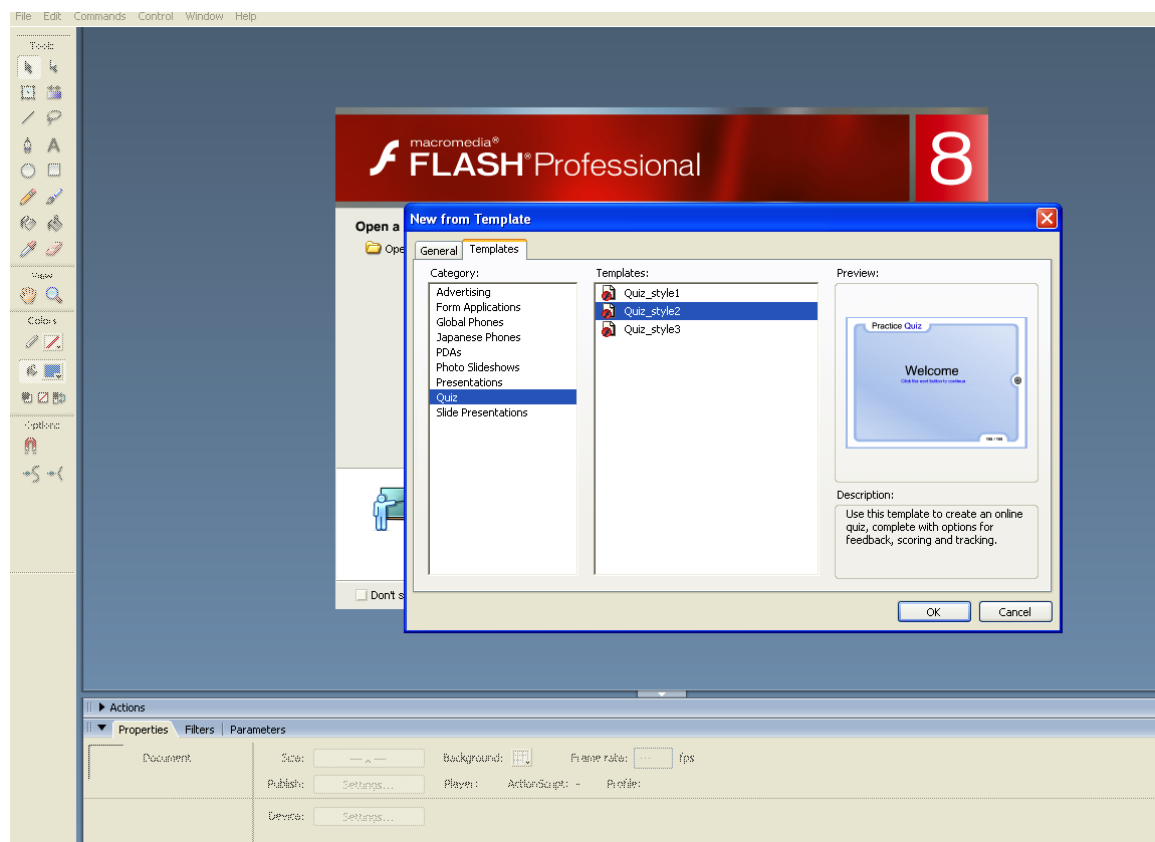
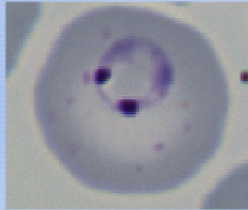

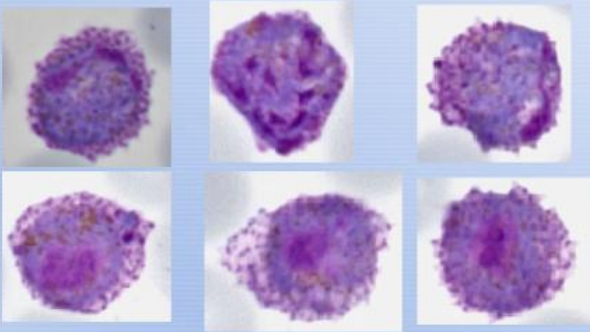


Figure 4.11: Inserting a flash quiz template

For each page there would be around five questions, but for pages with more information more questions were included.

There were six different types of quizzes that could be included shown in figure 4.12.

<p>Practice Quiz</p> <p>Which stage of the parasite life cycle is shown in this image?</p>  <ul style="list-style-type: none"> <input type="checkbox"/> Trophozoite <input type="checkbox"/> Gametocyte <input type="checkbox"/> Schizont <input type="checkbox"/> Accole form <p>Check Answer Click on a Checkbox.</p>	<p>Multiple choice</p> <p>Can be used with or without images</p>
<p>Practice Quiz</p> <p>Schizonts are rarely seen in <i>P. falciparum</i> infections</p> <ul style="list-style-type: none"> <input type="radio"/> A. True <input type="radio"/> B. False <p>Check Answer Click on a Radio Button.</p>	<p>b) True or false</p> <p>Can also be used for yes and no answers</p>

<div data-bbox="325 210 1121 790"> <h3><i>P. falciparum</i> Quiz</h3> <p>Click on the parasite</p>  <p>Check Answer Reset</p> <p>Click on an object to select it.</p> </div>	<p>c) Hotspot</p> <p>Can be used to select area in which parasite seen</p>
<div data-bbox="325 864 1121 1444"> <h3>Practice Quiz</h3> <p>Which of these is the odd one out?</p>  <p>Check Answer Reset</p> <p>Click on an object to select it.</p> </div>	<p>d) Hot objects</p> <p>Select the object that shows the correct answer</p>

	<p>e) Drag and drop</p> <p>Move the text over the target</p>
	<p>f) Fill in the blank</p> <p>The correct answer will need to be in the correct case to be accepted</p>

Figure 4.12: Examples of the different quiz frameworks available to be used in flash, a) Multiple choice, b) True or false, c) Hotspot, d) Hot objects, e) Drag and drop, f) Fill in the blanks

To edit each quiz once it was added, the component inspector (figure 4.13) was used to edit the content of the quiz and also be determine what the correct answer was.

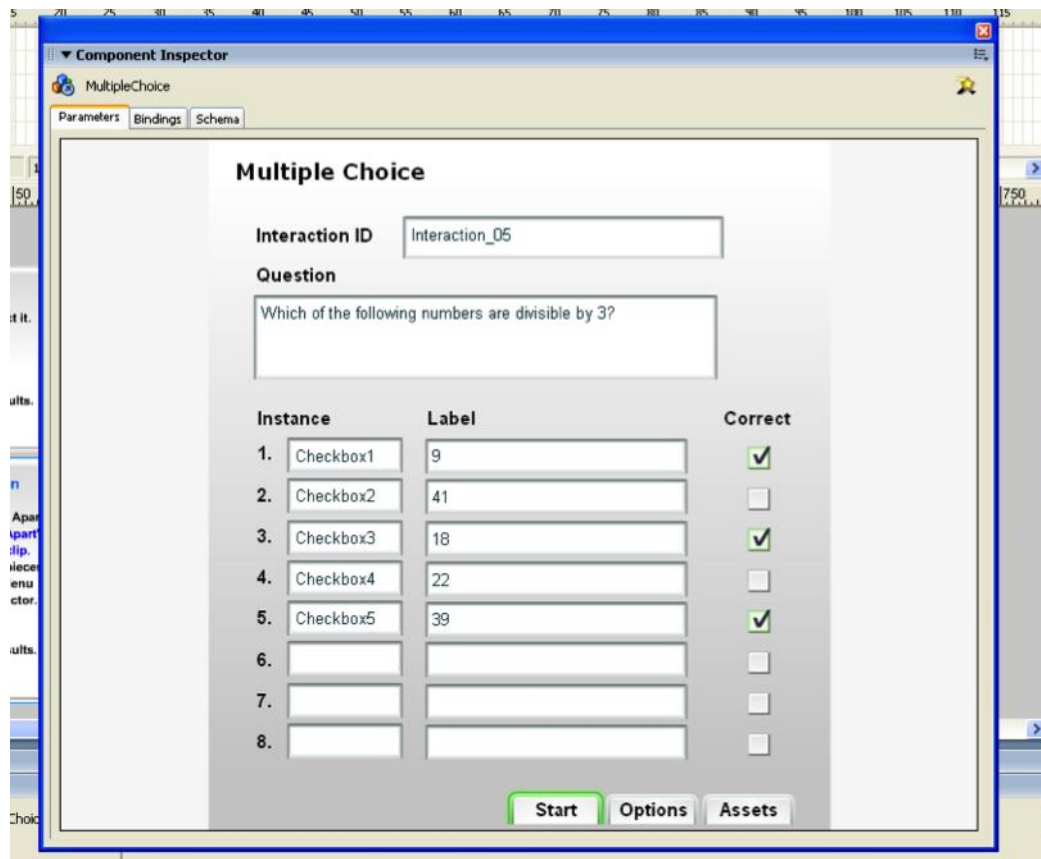


Figure 4.13: The component inspector window, allows the question to be added and the correct answer to be chosen
The participants were given a score at the end of the quiz to allow them to monitor progress (figure 4.14).

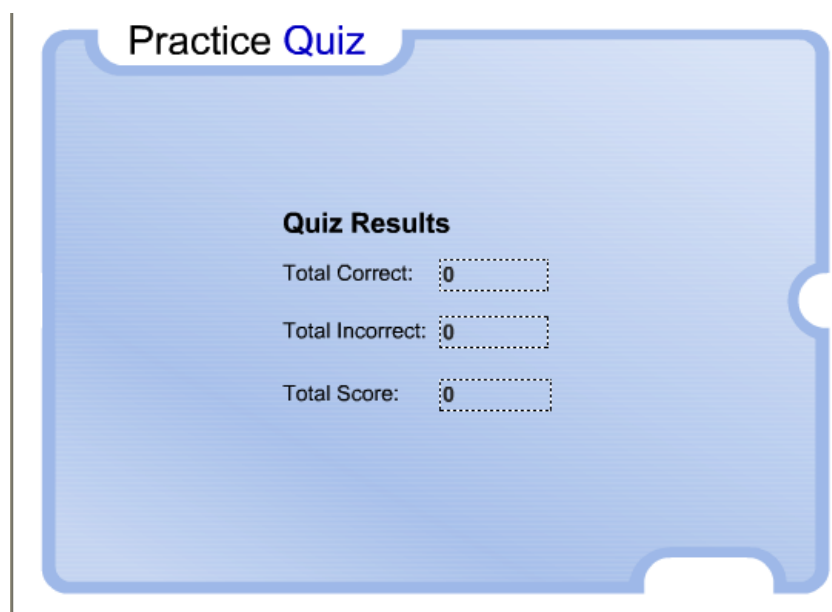


Figure 4.14: The participant score shown in the final screen
4.6.3 Results

The format of these quizzes did not allow feedback to be given immediately, the participants were provided with a yes or no answer when they clicked on the check answer button and a score at the end. However, this does not encourage learning, the participants should be able to see the correct results immediately after submitting, with an explanation of the result.

To allow immediate feedback to be given, frames were inserted between the quizzes, with an explanation of the answers. However, once this page was inserted the quiz did not operate in the same way. After some investigation and discovering how the template worked, a new control button was added, allowing the participants to move between the questions (figure 4.15). However, this allowed the participants to skip the question without answering it, but no alternative mechanism was discovered in the time available.

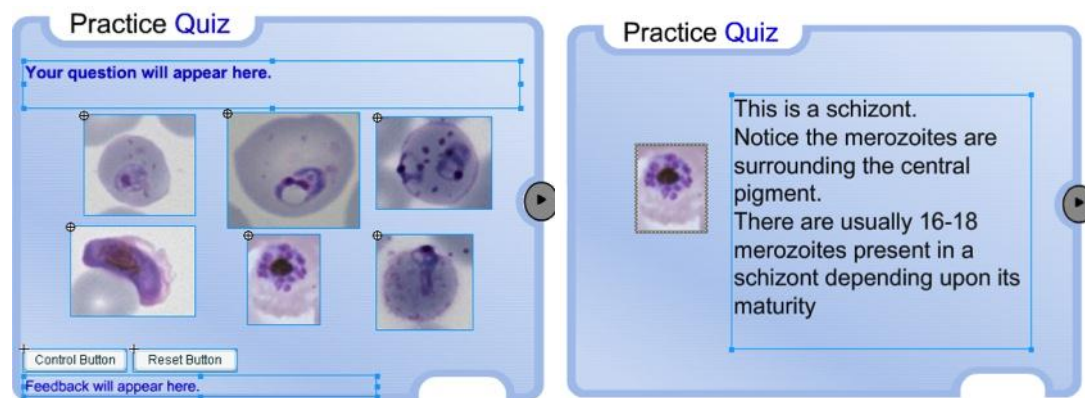


Figure 4.15: The question page provided on the left with the feedback page on the right
To enable the quiz to be placed within the webpage, initially it had to be published into a SWF file (figure 4.16).

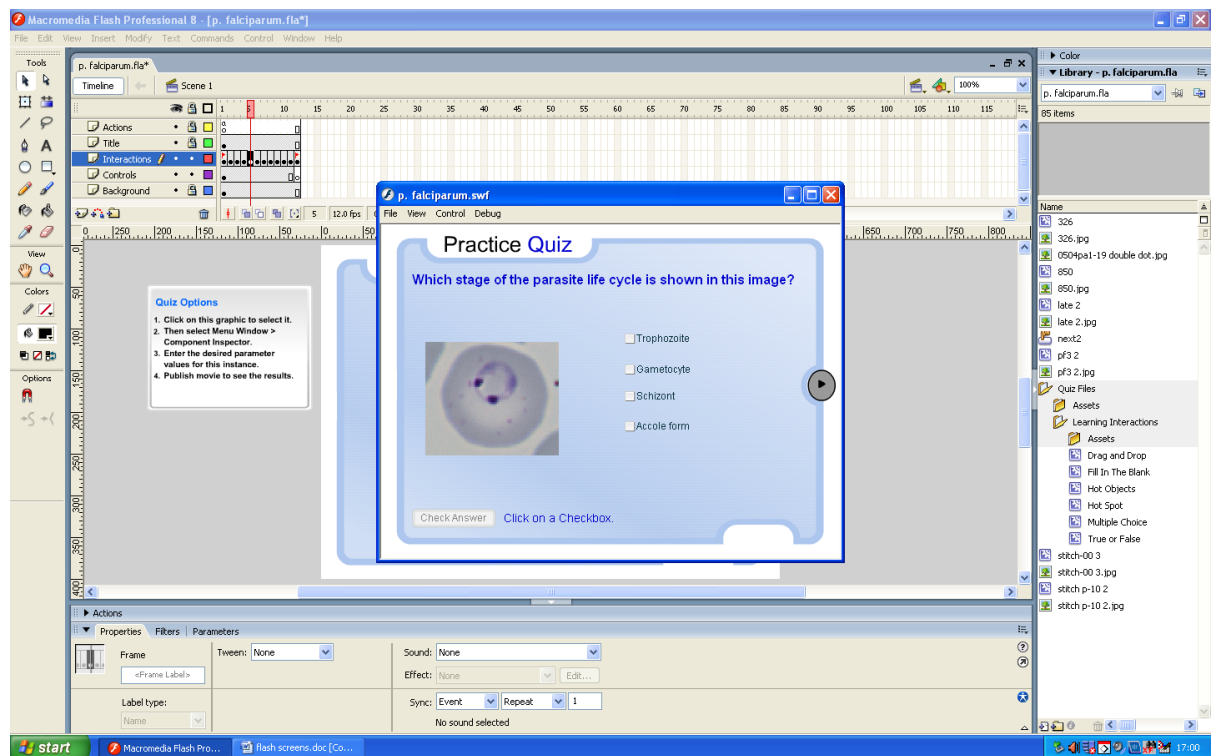


Figure 4.16: Publishing of the SWF file in Flash Professional
The SWF file was then inserted into the basic webpage format, with all of the relevant links present (figure 4.17).

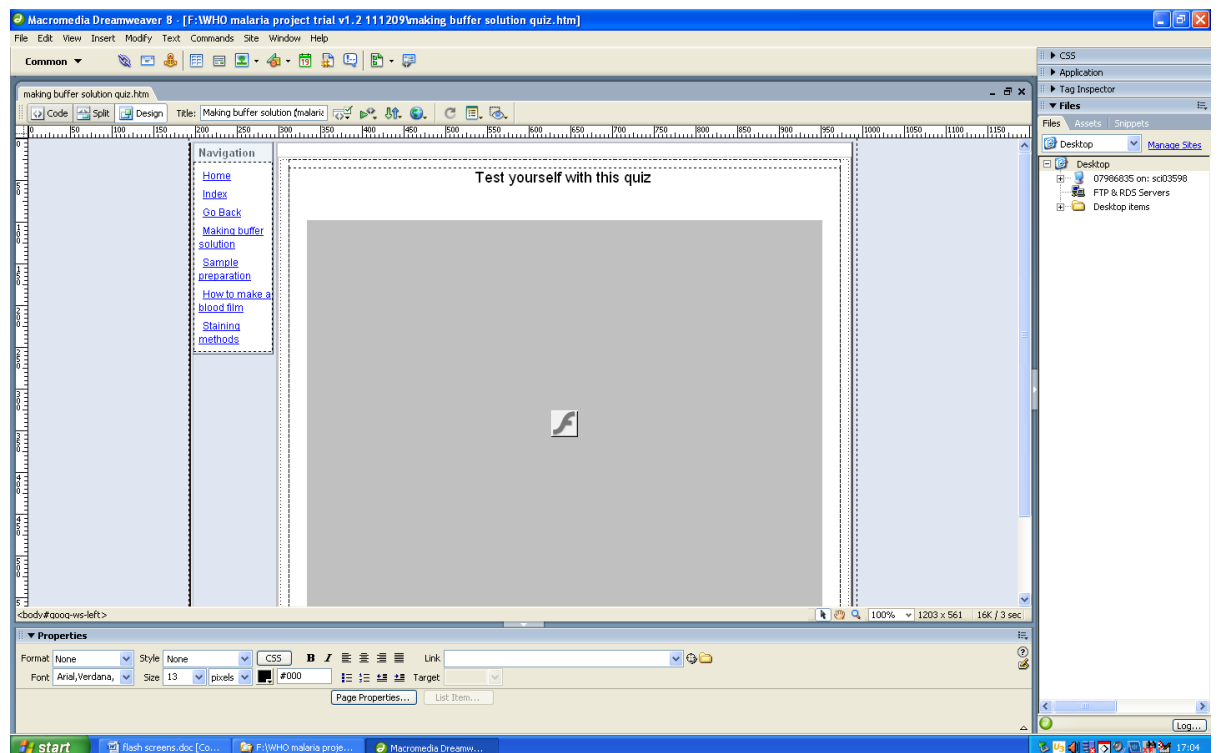


Figure 4.17: The final quiz file inserted into the HTML page as an SWF file

4.6.4 Discussion

Interactive quizzes with immediate feedback are required to engage participants with the training programme. The quizzes generated used Adobe Flash, which was also required to view the virtual microscope, therefore this did not require any additional equipment. The quizzes were added to the bottom of each page, allowing the participants to test their knowledge of what they had already learnt, and highlight areas that needed further development. Quiz templates were used in combination with images to enhance learning.

4.7 Generating images for atlas galleries

4.7.1 Introduction

To generate the image gallery a number of different blood smears and cells were photographed. The images generated were of a range of samples from ideal to those that were less obvious and showed some storage changes. These were used to allow the individuals to use these to identify cells on their own microscope. The quality of the image was dependent upon the preparation of the microscopic slide used and not the microscope.

Delivery of images

There was a number of way in which images could be delivered, which were investigated, listed as follows.

- Drawn images
- Microscopic pictures
- Atlas gallery
- Individual images
- Comparison tables
- Cell and description

All of these methods were used to different degrees throughout the project.

4.7.2 Methods

Individual images were taken with the Zeiss Axiolmager M1 microscope as described in section 3.3.2. The microscope was initially set up using the live properties (figure 4.18) window in the live view.

To achieve a high quality image a number of steps had to be carried out using the live properties tab. Once the image had been focused the following adjustments were made

- Light settings- the measure button on the adjust tab, was used to detect the correct light exposure required.
- Colour settings- to achieve the correct colour initially the white balance button was used (figure 4.18) followed by the interactive tool, on the adjust tab which was placed in a white area between cells. The dropper was placed where the red, green and blue readings were as close as possible and then pressed.
- Even background- to achieve an even background the slide was moved off the smear to a clear area with no stain deposit, the shading correction button on the general tab was used.
- Camera settings- the camera was set to 12MPx and the high quality setting found in the frame tab of the live properties window.
- Histogram- The curve of the histogram was adjusted to achieve the correct brightness and contrast settings, to achieve an image as close to that seen down the microscope eye pieces as possible.

Images were taken using the snap button shown in figure 4.18. The image is then left on the desktop and is required to be saved before closing the file. The files were saved as TIFF files to allow processing to generate the gallery images.

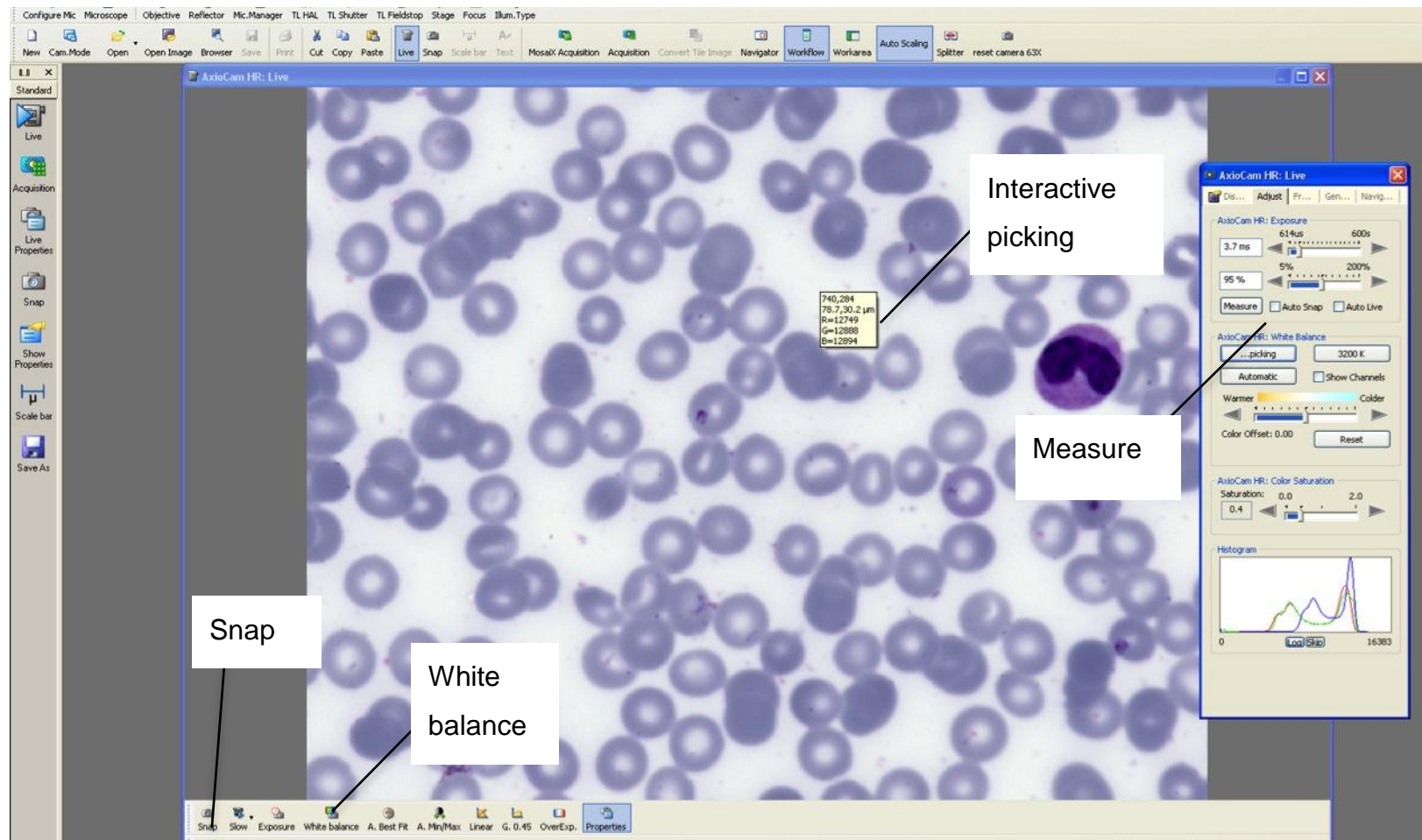


Figure 4.18: The live view window showing the properties window and settings that can be adjusted

4.7.3 Results

Different camera resolution settings were evaluated to determine which resolution was the best to use for the gallery images (figure 4.19). There were three resolution settings available

- 1.2 MPx (1300 x 1030 pixels)
- 5 MPx (2600 x 2060 pixels)
- 12 MPx (3900 x 3090 pixels)




	1.2 MPx
	5 MPx
	12 MPx

Figure 4.19: Comparison of images at different resolutions

The image from the 12 MPx camera can provide the same resolution as a 100X lens.

4.7.4 Discussion

Images were generated with the 12 MPx camera on the Zeiss microscope to create the largest possible image to be used in the image gallery. The 12 MPx image was three times the size of the 1.2 MPx image due to the greater number of pixels present. When placing the image into the gallery the size of the image was then adjusted to ensure that there was no pixilation. The image all saved as TIFF files, but were converted to JPEG images after they had been processed.

4.8 Processing images for atlas galleries

4.8.1 Introduction

To ensure that the images viewed over the Internet were of the same quality as those seen down the microscope, a few image correction stages were carried out. Image enhancement was restricted to revealing detail and enhancing focus. To ensure images had a natural “microscope” appearance care was taken at the capture stage to avoid over enhancement of contrast, producing a bleached background.

4.8.2 Methods

Cropping the image

Only a small area of the image captured was required for the photo gallery. To obtain the size of image required Adobe Photoshop was used to reduce the size of the original image. Before modifying the original file a duplicate was saved under a different name. This file was then reduced to the required size using the crop tool.

Detail enhancement

Detail enhancement allows features that may not be as defined as they are in the microscope image to be improved. The settings for this were explored before using them in the images generated.

Initially the software to be used for the detail enhancement was chosen, an Adobe Photoshop add-in Digital Outback Photo -Detail Extractor Version 2 was chosen through experimentation and comparison to other add-ins available at a similar cost. The same settings were used as described in section 3.4.4.

Different settings for these were also explored, varying these to achieve the best image.

Contrast mask

The contrast mask settings darken the image to even out the colour enabling the image to be more representative of the original. This process allows better contrast between the background and the cells, allowing them to be seen more easily and reduced noise in the background.

Smart sharpen

Smart sharpen allows particular colour settings to be processed to allow the image to be of the optimal quality. The following smart sharpen settings were used

- Convert to 16 bit (image menu)
- Choose smart sharpen (in filter menu)
 - Amount 125%
 - Radius 11 pixels
 - Angle 0
 - With more accurate

- Remove: Lens blur
- Shadow: Parameters
- Amount 5%
- Tone width 80%
- Radius 10
- Highlight: Parameters
- Amount 20%
- Tone width 70%
- Radius 70
- Convert to 8 bit

The files were saved as TIFF files after the process was completed.

4.8.3 Results

Detail enhancement and contrast mask

These settings were explored in section 3.4.4.

Smart sharpen

Figure 4.20 compares the original image, detail-enhanced image and smart sharpen image.

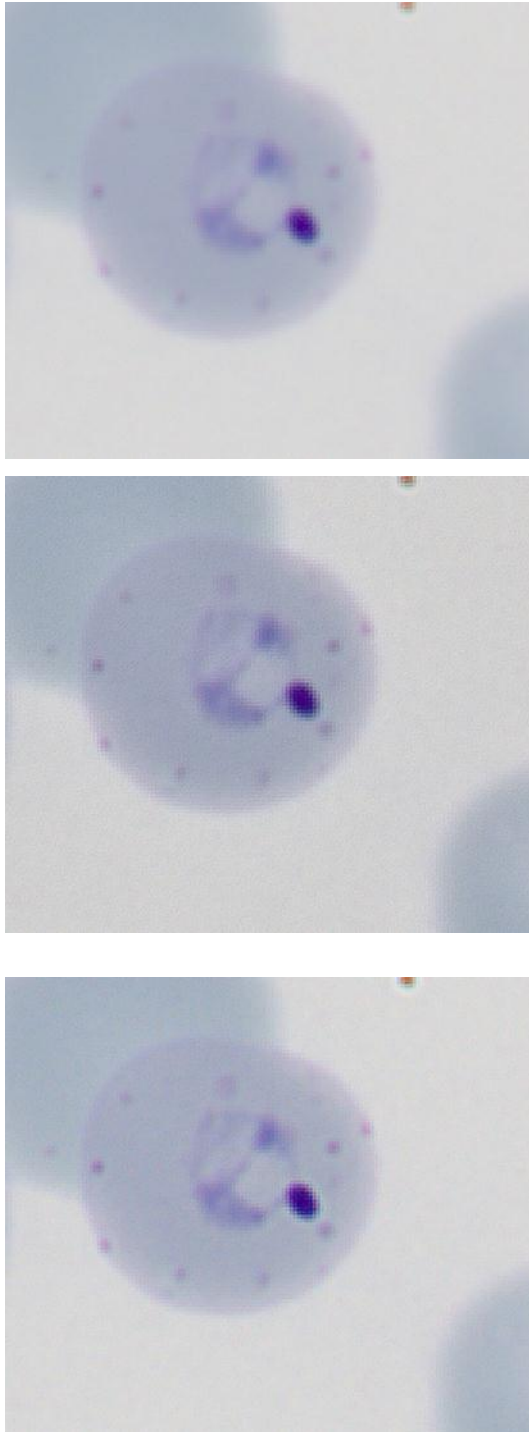


Figure 4.20: Comparison of images generated using the different methods. Top left- original file, top right detail enhanced image, bottom smart sharpened image

4.8.4 Discussion

Smart sharpened images shown in figure 4.20 were deemed to give the best quality image and were therefore used for all the photo gallery images. The images were processed directly once the file had been cropped to the required size. This process was also carried out on the single shot images

linked to from the gallery to make sure the participants viewed the same image.

4.9 Review of the training programme

4.9.1 Introduction

The author solely developed the training programme, with input from the supervisory team, however it was felt expert review was needed to ensure the information given was “fit for purpose”.

4.9.2 Method

The content of the training programme was initially reviewed within the digital morphology team at Manchester Royal Infirmary and edits were made. Following these edits members of the UK NEQAS Morphology Specialist Advisory group made comments. The training and a questionnaire was distributed on a USB stick to

- Educationalists (1)
- Consultants (5)
- Biomedical Scientists (10)
- Trainees/ students (1)

Seventeen USB sticks were distributed in total, eleven of which were returned. The questionnaire distributed can be seen in appendix 1.1.

The comments made by these individuals were then incorporated into the final version of the programme. Some errors in the text were corrected as well as additional information added. Some of the page formats were also altered to improve understanding.

4.9.3 Results

Table 4.1: Results from the training programme review questionnaire

	Yes	No	Not answered
Easy to access	9	1	1
Sidebar appropriate	6	3	2
Content appears correctly	11	0	0
Enough detail present	10	1	0
All expected information present	11	0	0
Information accurate	9	2	0
Images satisfactory quality	8	2	1
Gallery images give accurate representation	10	0	1
Mechanism logical	9	1	1
Would you approach delivery differently?	5	5	1

To check that the content of the training was deemed to be appropriate and contained enough information. The results of the questionnaire are shown in table 4.1, with the questionnaire being shown in appendix 1.1.

Alongside the questionnaire the individuals also made comments and highlighted points that needed amending. Changes were suggested to parts of the structure as some of the images were not of the correct quality. For

example, one individual noted that links should be made to more detailed pages at the end of each section to make links clearer. On the stages of malaria infection page the link to the trophozoite page was placed after the description, in a separate sentence. Comments upon changing the approach included further description of the features of each species, rather than by stage; giving a more practical approach; adding diagrams to reduce text and adding more colour. These were addressed where possible within the basic html format used,

4.9.4 Discussion

Following the results of the questionnaire, the suggested amendments were made. The order of delivery was changed in some places to make the links more relevant. For example, links to the galleries were moved to the bottom of the text section on the trophozoites page, to prevent confusion with other links within the text.

At the time of the review the planned quizzes were not included, however a number of individuals suggested that quizzes should be added. The thick film information had also not been added at this stage, with many individuals saying that it was necessary.

Some individuals had problems accessing the sidebar, which was amended. Many found they lost where they were in the project, an index page was therefore added to enable participants to determine which pages they had or had not visited. The links on the sidebar were also changed, to allow individuals to link easily between relevant pages.

All comments received were positive and many showed the training to other colleagues or requested a copy of the final version.

Chapter 5: Results for the International and UK groups

5.1 Participants recruited onto the intervention study

5.1.1 International group participants recruited

Participants were recruited from four different African countries Ghana, Kenya, Malawi and Nigeria, and in Chile, Colombia, Hong Kong, India, and Lebanon (figure 5.1). Fourteen laboratories were recruited, six of these in Nigeria, where Internet connectivity was relatively poor. Forty-two participants were recruited onto the project.

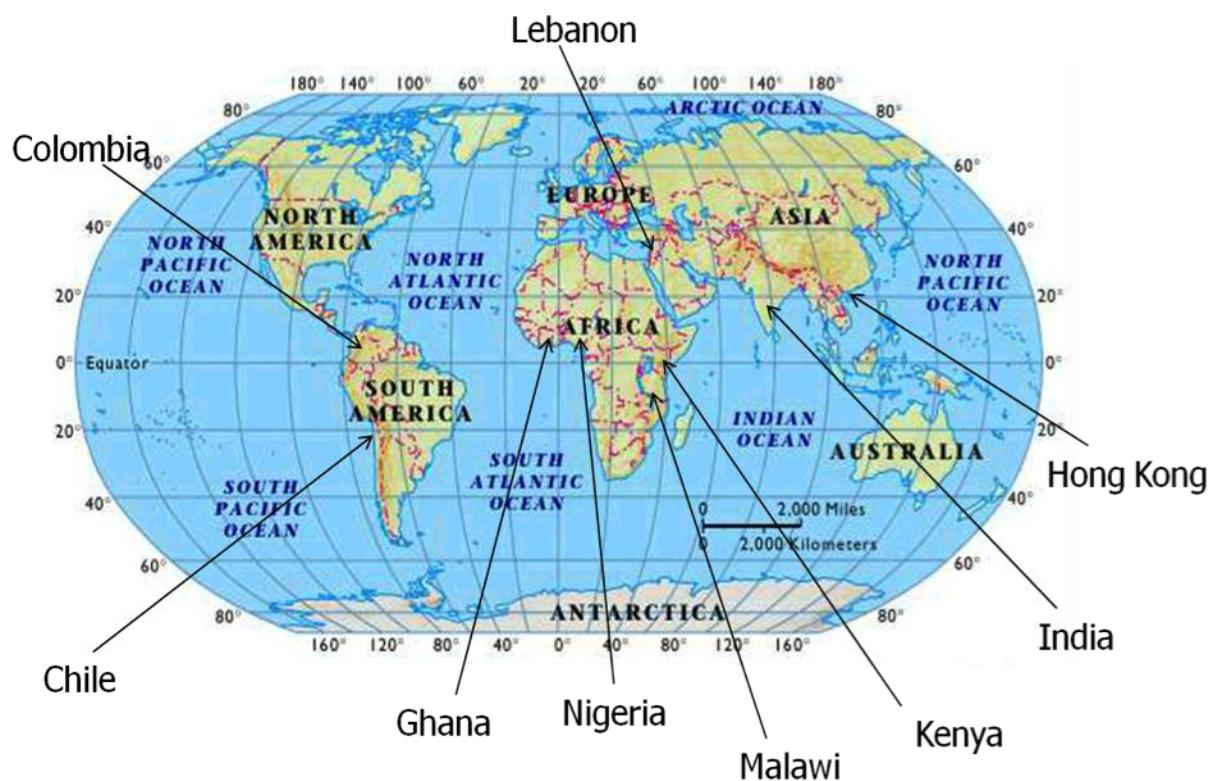


Figure 5.1: Locations of participants around the world

Within Nigeria the six laboratories were based in three different cities (figure 5.2). Lagos is on the South West coast of Nigeria, Ibadan also in South West Nigeria, about 50 km further inland from Lagos and, Kano is in Northern Nigeria.



Figure 5.2: Map of Nigeria, participants were in Lagos, Ibadan and Kano (Global alliance, 2001)

The names of the laboratories involved were anonymised, and assumed the following nomenclature

- Ibadan 1 (Hospital laboratory)
- Ibadan 2 (Research laboratory)
- Lagos 1 (Hospital laboratory)
- Lagos 2 (Hospital laboratory)
- Lagos 3 (Private laboratory)
- Kano (Hospital laboratory)

The Kenyan laboratory was located in Nairobi. The location of the other laboratories within their country was unknown, as they were provided to the author anonymously by the WHO and UK NEQAS.

5.1.2 UK group participants recruited onto intervention study

Participants were recruited initially by contacting laboratory managers at random from a list of laboratories that participate in the UK NEQAS scheme for parasite identification. Initially ten individuals were contacted by telephone, and then subsequently by email giving further details. All the members of staff contacted were then asked to provide the names of individuals within their laboratory who would be included in two specific staff categories.

- <2 years experience, and pre-specialist portfolio
- >5 years experience, and post-specialist portfolio

These categories were used to see if the information in the training programme required any precursor knowledge, or whether there was not enough information for the more experienced individuals. The groupings were used to analyse results achieved in the initial and final assessment.

Interested participants were asked to complete an online questionnaire to record their professional background and training experience. An outline of the questions asked can be seen in Appendix 1.4. These participants were then given access to the training website to access the images. 39 participants were asked to complete the questionnaire, 33 did so, 34 participants commenced the study by accessing the images.

5.2 Delivery of the intervention training programme

Due to the increased availability of Internet access for this group in the UK, the time scales of the project were shortened from those in the international group. The participants were given access to the initial assessment images over a six-week period. The images were released in sets of ten, with a set released each week for the first four weeks, and all available for the last two weeks. The training was then delivered over a six-week period, with a final six weeks for the final assessment.

5.3 Results from the initial recruitment questionnaire

5.3.1 International group results from the recruitment questionnaire

Upon recruitment, participants were asked to complete a questionnaire about their personal experience and training (appendix 1.3). The laboratory coordinator also completed a questionnaire about the laboratory and the techniques used in malaria diagnosis.

Laboratory questionnaire

Number of staff

The number of staff present within the laboratory was dependent on the number of tests requested, the location of the laboratory and its size. The majority of laboratories had 5-6 members of scientific staff (n=7) although there were also laboratories with 1-2 (n=1) and more than 15 (n=1) also recruited. In most laboratories there were five or more members of staff carrying out malaria microscopy (n=10). There were however, two laboratories with only one member of staff diagnosing malaria.

Number of malaria cases

Twelve laboratories reported seasonal variation in the number of malaria cases seen. In the high season the majority of laboratories saw 30-49 cases (n=6) per week, there were four laboratories that saw more than 50 cases per week. In the low season 10-29 cases were most frequently reported (n=7), there was one laboratory reporting more than 50 cases, this was not the laboratory with the highest number of staff however. The parasite density was reported to be between one and 8% by ten of the laboratories. There was one laboratory reporting parasite densities of more than 8% in the majority of cases.

Microscopes

The majority of laboratories had two microscopes for malaria diagnosis (n=7), three laboratories had more than five. In the 14 laboratories questioned there were a total of seven non-functioning microscopes and more than 40 functioning microscopes,

in broad agreement with the findings of (Mundy *et al.*, 2000). The number of microscopes therefore influences the number of cases that can be examined, especially in the high season.

Staining techniques

The microscope slides for malaria were commonly stained with Giemsa stain with the thin film being fixed with methanol. However, the methanol was not changed more often during the wet season, potentially leading to an increase in poorly prepared microscope slides. Methanol allows fixation of the cells to the slide, the presence of water can prevent fixation of the cells, and also lead to artefactual changes. The artefactual changes include rings within the erythrocytes, which can look like parasites causing false positives, or false negatives if this overlaid a parasite, making it impossible to see.

Power supply and computer access

Of the respondents ten reported that electricity was usually reliable. Ten of the laboratories questioned had their electricity supplied by generator for 24 hours of the day, one for 12, and one for six hours or whenever enough fuel was available.

Half of the laboratories questioned had access to computers in the laboratory, six of these laboratories had Internet access. Those laboratories that did not have a computer in the laboratory or did not have Internet access were required, for the study, to visit Internet cafes. Financial support was provided to allow access for these individuals.

Questionnaire completed by laboratory based individuals

Figure 5.1 shows some of the international participant responses to the questionnaire.

Table 5.1: International participant recruitment questionnaire results

Participant	Time at this laboratory (years)	Malaria diagnosis experience (years)	Education for malaria diagnosis	Time since last training (years)
LT001	1-4	1-4	External training course	<1
LT002	<1	1-4	Post-graduate qualification	<1
LT003A	1-4	5-9	Diploma	1-4
LT003B	1-4	1-4	Diploma	<1
LT004	1-4	1-4	External training course Diploma	1-4
LT005	5-9	5-9	External training course Diploma	1-4
LT006A	≥10	≥10	Diploma Post-graduate qualification	≥10
LT006B	5-9	5-9	External training course	<1
LT006C	<1	5-9	Post-graduate qualification	5-9
LT006D	≥10	≥10	External training course Post-graduate qualification	≥10
LT006E	1-4	5-9	External training course Diploma	<1
LT006F	5-9	<1	Diploma	<1
LT007	1-4	1-4	Diploma	<1

Table 5.1: International participant questionnaire results

Participant	Time at this laboratory (years)	Malaria diagnosis experience (years)	Education for malaria diagnosis	Time since last training (years)
LT008	1-4	1-4	External training course Diploma	<1
LT009	1-4	1-4	Diploma	<1
LT010	1-4	1-4	External training course	NR
LT011	≥10	≥10	Post-graduate qualification	NR
LT012	≥10	≥10	External training course	<1
LT013	1-4	1-4	External training course Diploma	<1
LT014	1-4	1-4	Other	1-4
LT015	1-4	1-4	External training course Diploma	<1
LT016	5-9	5-9	Post-graduate qualification	1-4
LT017	≥10	≥10	External training course	1-4
LT018A	5-9	5-9	External training course	NR
LT018B	1-4	1-4	External training course	NR
LT018C	<1	<1	External training course	NR
LT018D	5-9	<1	External training course	NR
LT018E	5-9	5-9	External training course	NR
LT018F	<1	1-4	Post-graduate qualification	NR

Table 5.1: International participant questionnaire results

Participant	Time at this laboratory (years)	Malaria diagnosis experience (years)	Education for malaria diagnosis	Time since last training (years)
LT019	1-4	1-4	Diploma	<1
LT020	1-4	1-4	Diploma	<1
LT021	1-4	1-4	Diploma	<1
LT022	1-4	1-4	Diploma	<1
LT023	1-4	1-4	External training course Diploma	<1
LT024	1-4	1-4	Diploma	<1
LT025	1-4	1-4	External training course Diploma	<1
LT027	1-4	5-9	External training course Diploma	1-4
LT028A	≥10	≥10	External training course Other	5-9
LT028B	1-4	1-4	External training course Other	1-4
LT028C	<1	<1	External training course Other	<1
LT028E	1-4	5-9	External training course Other	1-4
LT028F	1-4	5-9	External training course Other	1-4

NR= No response

Table 5.1: International participant questionnaire results

Experience

The majority of participants (n=21) had 1-4 years experience. Six participants had more than ten years experience in diagnosing malaria. Conversely, four participants had less than one year experience at the time the questionnaire was administered.

Qualifications of the laboratory staff

The majority of participants had completed a degree or diploma (n=23) or a combination of both to enable them to take up their post. Seven had completed a postgraduate qualification and/or alternative examinations to demonstrate competency to practice.

Training of the laboratory staff

Most participants (n=26) had completed an external training course to learn how to diagnose malaria, but training from other staff in the laboratory was also common in some laboratories (n=20). In addition most laboratory staff (n=6) underwent specific training in the diagnosis of malaria provided by local recognised providers, which included staining techniques and identification of malaria species.

The international participants were also asked when they last received training. Only 34 of the participants responded to this question, with 20 saying they had received training within the last year.

Methods used in the diagnosis of malaria in the participating laboratory

Participants were asked to record the methods used within their laboratory and how parasite density was calculated. The majority of laboratories questioned used both the thick and thin films. Therefore, participants gave an answer for the thick and thin film. All participants used the RBC method on the thin film to calculate the percentage parasite density. On the thick film the method used varied, most laboratories used the WBC method, but others used a rank system, ranking the number of parasites into one of four groups (+, ++, +++, ++++).

5.3.2. UK group results from the recruitment questionnaire

The questionnaire results of the UK group are shown in table 5.2.

Table 5.2: The UK participants response to the recruitment questionnaire and their locations and experience

ID code	Report blood films	Experience	Portfolio	Role	IBMS	Parasitology training	Number of malaria cases	Location
UK101	Yes	1-2	Studying specialist	Specialist BMS	Licentiate	No	16-25 cases	4
UK111	Yes	6-10	Specialist	BMS registered	Member	Yes 2005	16-25 cases	4
UK121	Yes	2-5	Studying specialist	BMS registered	Licentiate	Yes 2006	6-15 cases	4
UK131	Yes	1-2	Studying specialist	BMS registered	Licentiate	No	<5	5
UK141	Yes	1-2	Studying specialist	BMS registered	Licentiate	Yes 2008	6-15 cases	5
UK151	No	1-2	Studying registration	Trainee	No	No	<5	5
UK161	Yes	>20	Studying diploma	Senior BMS	Fellow	No	<5	1
UK171	Yes	>20	Studying higher	Specialist BMS	Member	Yes 2009	6-15 cases	1

Table 5.2: The UK participants response to the questionnaire and their locations and experience

ID code	Report blood films	Experience	Portfolio	Role	IBMS	Parasitology training	Number of malaria cases	Location
UK181	Yes	6-10	Higher specialist	Specialist BMS	Member	Yes 2007	6-15 cases	1
UK191	Yes	>20	N/A	Senior BMS	Fellow	Yes 1988/99	6-15 cases	1
UK201	No	<1	Studying registration	Trainee	No	No	NR	1
UK211	Yes	>20	N/A	Senior BMS	Fellow	Yes 1993	6-15 cases	3
UK221	Yes	6-10	Higher specialist	Specialist BMS	Member	No	36-45 cases	3
UK231	Yes	1-2	Studying specialist	BMS registered	Licentiate	No	26-35 cases	3
UK241	Yes	6-10	Studying specialist	BMS registered	No	No	16-25 cases	3
UK251	Yes	6-10	Studying diploma	Senior BMS	Fellow	Yes 2001	6-15 cases	6
UK261	Yes	>20	Higher specialist	Specialist BMS	Fellow	No	6-15 cases	7

Table 5.2: The UK participants response to the questionnaire and their locations and experience

ID code	Report blood films	Experience	Portfolio	Role	IBMS	Parasitology training	Number of malaria cases	Location
UK271	Yes	>20	N/A	Senior BMS	Fellow	Yes 1998	6-15 cases	8
UK281	Yes	6-10	Higher specialist	BMS registered	Licentiate	Yes 2008	6-15 cases	8
UK301	Yes	2-5	Studying specialist	BMS registered	Licentiate	No	6-15 cases	8
UK311	Yes	2-5	Studying specialist	BMS registered	Licentiate	No	6-15 cases	8
UK331	No	1-2	Studying specialist	BMS registered	Member	No	6-15 cases	6
UK341	No	1-2	Studying specialist	BMS registered	Licentiate	No	NR	6
UK361	Yes	6-10	Studying specialist	BMS registered	Licentiate	No	6-15 cases	2
UK371	Yes	16-20	Higher specialist	Senior BMS	No	Yes 1997	6-15 cases	2
UK381	Yes	16-20	N/A	BMS registered	No	Yes 2004	16-25 cases	2

Table 5.2: The UK participants response to the questionnaire and their locations and experience

ID code	Report blood films	Experience	Portfolio	Role	IBMS	Parasitology training	Number of malaria cases	Location
UK391	Yes	>20	N/A	BMS registered	No	Yes 2009	16-25 cases	2
UK401	Yes	6-10	N/A	BMS registered	No	Yes 2000	16-25 cases	2
UK411	Yes	2-5	Studying specialist	BMS registered	Licentiate	Yes 2007	16-25 cases	2
UK431	Yes	2-5	Studying specialist	Specialist BMS	Licentiate	Yes 2008	6-15 cases	2
UK441	Yes	>20	Specialist	BMS registered	Fellow	No	6-15 cases	2
UK461	Yes	>20	N/A	BMS registered	No	No	16-25 cases	2
UK471	Yes	>20	N/A	Senior BMS	No	Yes 1995	16-25 cases	2
UK481	Yes	16-20	Higher specialist	Specialist BMS	No	Yes 2004	<5	2

NR= no reply, N/A= not applicable

Table 5.2: The UK participants response to the questionnaire and their locations and experience

The questionnaire for the UK group was used to obtain detailed professional information about the individuals involved in the project. The responses initially split the individuals into two categories as described above. Of the responders there were 15 individuals in category one (<2 years experience) and 18 in category two (>5 years experience).

51.5% of participants had taken part in the UK NEQAS parasitology training scheme.

5.4 Initial assessment

5.4.1 International group

In the initial assessment, the participants of the international group were provided with 40 microscopic images, and were given the opportunity to provide a diagnosis for each case.

Appendix 1.2 describes the details of each of the digital blood smears used in the initial assessment, giving the diagnosis and features that may affect diagnostic accuracy.

Of the 42 participants, 24 participants completed all 40 cases within the allocated time. Another 15 completed various cases (n=3-31) throughout the project. The results of the initial stage were used to assess the competency of diagnosis of the laboratory staff involved in the day-to-day diagnosis of malaria. The results of each specimen are shown in table 5.3.

Table 5.3: The detection of parasites in the initial assessment stage slides (n=40) for the international participants group.

Definitive diagnosis	n (=40)	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)
Negative	7	90.9	11.6	NA	NA
<i>P. falciparum</i>	24	74.5	82.8	53.2	87.2
<i>P. vivax</i>	3	81.8	48.5	35.0	42.4
<i>P. ovale</i>	4	47.4	54.2	6.6	20.7
<i>P. malariae</i>	1	17.2	NA	13.8	NA
Mixed infection	1	89.7	NA	3.5	NA

Species detection accuracy- the ability of the microscopists to make the correct diagnosis, identifying the presence or absence of parasites.

Species identification accuracy- the ability of the microscopist to identify the correct species of malaria parasite present in the blood film.

Overall, the diagnosis of malaria for all international participants across all 40 cases gave a detection accuracy of 74.6% (± 29.3). However, in no single case was the outcome correctly diagnosed by all participants in terms of determining the presence of parasites and in identifying correct species. There were eight cases in which all participants correctly identified the presence or absence of parasites.

The species detection accuracy was low at 43.7% (± 28.1). One case had the correct species identified by all participants, who had identified the presence of parasite density. The species identification accuracy was highest for *P. falciparum* cases, which all participants from this international group see on a daily basis.

Table 5.4 demonstrates performance on the individual cases in the initial assessment by the international group.

Table 5.4: Performance on the individual cases (n=40) in the initial assessment by the international group

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
1	<i>P. falciparum</i>	35.1	32.4	Yes	2	3	1
2	<i>P. ovale</i>	34.2	2.6		1	2	2
3	Negative	85.7	-		-	4	2
4	<i>P. falciparum</i>	91.4	74.3		1	3	2
5	Negative	85.3	-		-	4	2
6	<i>P. falciparum</i>	100.0	91.2		3	0	1
7	Negative	85.3	-		-	3	1
8	<i>P. vivax</i>	100.0	38.2		2	2	2
9	<i>P. falciparum</i>	97.1	73.5		3	1	1
10	<i>P. vivax</i>	51.5	12.1	Yes	1	3	3
11	<i>P. falciparum</i>	96.9	53.1		2	1	1
12	<i>P. falciparum</i>	100.0	78.8		3	0	1
13	<i>P. falciparum</i>	100.0	72.7		3	0	1
14	<i>P. falciparum</i>	100.0	45.5		3	2	1

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
15	<i>P. falciparum</i>	90.9	69.7	Yes	2	2	2
16	<i>P. falciparum</i>	87.9	63.6		2	1	1
17	<i>P. ovale</i>	33.3	3.0		1	0	1
18	<i>P. falciparum</i>	97.1	48.6		2	0	1
19	<i>P. falciparum</i>	100.0	94.1		3	1	1
20	<i>P. falciparum</i>	100.0	64.7		3	1	1
21	<i>P. falciparum</i>	44.1	38.2		1	2	2
22	<i>P. vivax</i>	93.9	54.6		2	0	2
23	Negative	93.8	-		-	1	2
24	<i>P. ovale</i>	87.5	0.0		1	1	2
25	<i>P. falciparum</i>	87.5	34.4		2	3	2
26	<i>P. falciparum</i>	100.0	68.8		2	2	2
27	Negative	96.9	-	Yes	-	0	2
28	<i>P. falciparum</i>	31.3	25.0		3	4	2
29	<i>P. falciparum</i>	21.9	18.8		2	4	2
30	<i>P. falciparum</i>	87.5	37.5		2	3	2

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
31	<i>P. falciparum</i>	76.7	76.7		1	4	3
32	<i>P. falciparum</i>	23.3	20.0		1	1	2
33	Negative	96.6	-		-	4	2
34	<i>P. falciparum</i>	17.2	6.9	Yes	2	4	3
35	Negative	93.1	-	Yes	-	4	3
36	<i>P. falciparum</i>	75.9	69.0	Yes	2	3	3
37	<i>P. falciparum</i>	42.9	35.7		1	4	3
38	<i>P. ovale</i>	34.5	20.7		1	2	2
39	<i>P. falciparum</i> and <i>P. ovale</i>	89.7	3.5		2	3	3
40	<i>P. malariae</i>	17.2	13.8		1	3	2
Average		74.6	43.7				

The main errors made allocated into three categories

- False negatives
- False positives
- Incorrect species

False negatives

False negative results were mainly due to one or more of the following:

Low parasite density

False negative results were reported on a number of low parasite density cases. For example, case 32, a thin film positive for *P. falciparum* with only one gametocyte present, was identified as negative by 23 out of 30 participants. Of the seven that identified the presence of parasite density, six made the correct diagnosis.

All 12 cases with a low parasite density had false negative results. The low parasite density *P. falciparum* cases 2 (25), 4 (3), 21 (19), 31 (7) and 37 (16), appear to have caused false negative results (number of negative cases reported in brackets). There were two low parasite density *P. vivax* cases; case 10, a thick film, was reported as negative by 16 participants, and case 22 was reported as negative by two participants. All three *P. ovale* cases were low parasite density cases; case 17 had 22 false negative results, case 24 had four and case 38 had 19. There was one *P. malariae* case, case 40, which had 24 of the international participants giving negative results.

Thick films obscuring parasites

There were seven thick blood films used in the initial assessment, and there were two negative films used. The use of thick blood films appeared to obscure parasites from the microscope user, even at higher parasite densities (number of negative cases reported in brackets), cases 1 (24), 10 (16), 15(3) 34 (24) and 36 (7) had high false negative rates.

Artefacts

There were five positive cases with an abundance of artefacts (rank 4). Cases 28 and 29 not only had high parasite densities but also had false negative results (22 and 25 respectively). In case 28, the parasites were faint and slightly out of focus, this combined with the stain deposit could have lead to the parasites being missed even at such high densities. Case 29 showed very small *P. falciparum* trophozoites in a patient that also had chronic granulocytic leukaemia, six correctly identified the *P. falciparum* infection. False negative results were also seen for cases 31 (7), 34 (24) and 37 (16) (number of negative cases reported in brackets).

False positives

False positives were seen mainly on cases with artefacts present, which could be confused with parasites present.

There were four negative cases with numerous artefacts present (rank 4). Case 3 showed five false positive results amongst the participants, possibly due to the artefacts present. These artefacts include platelets overlying erythrocytes and stain deposits overlying the erythrocytes.

Cases 5 and 7 showed five false positive results. Case 7 showed intense basophilic stippling found in other haematological disorders, such as heavy metal poisoning. The basophilic stippling has a similar appearance to the stippling seen in *P. vivax* and *P. ovale* infection but without the parasite within the cell.

Incorrect species

The identification of the species present caused more difficulty than identifying the presence of parasite density, especially in cases other than *P. falciparum*, which is most commonly seen in the study regions involved. The species identification accuracy in cases 2, 17 and 24 was poor, these were all cases with *P. ovale* infection. There were also problems with *P. vivax* identification, case 8 had the incorrect species identified by 25 participants, case 10 by 13 and case 22 by 13. A large number of participants did not correctly identify the late trophozoites and recorded these infections as *P.*

falciparum infection. This is clinically significant as the treatment differs between these two species.

Low parasite density

There were six low parasite density *P. falciparum* cases used in the initial assessment, in five of which difficulties in species determination were observed. Case 2 was identified as a different species by four participants, case 4 by six, case 21 by two, case 32 by one and case 7 by two. These difficulties were probably caused by a lack of parasites present in the specimens.

Thick films

All of the four *P. falciparum* thick films used caused difficulties in species identification. Case 1 had one participant who identified this specimen as *P. vivax*, case 15 was identified as *P. vivax* by four participants and three identified it as *P. malariae*. Case 34 had three incorrect species determinations, and finally, case 36 had two, one *P. ovale* and one *P. malariae*.

Artefact

Little influence on species determination was seen in the five cases, where numerous artefacts were present (rank 4). Cases 28 (2), 29 (1), 34 (3) and 37 (2), caused more difficulties in detection than in species determination (figures in brackets indicate number of incorrect species).

Cell inclusions

Cases 6 (3), 9 (8), 11 (14), 12 (7), 13 (9), 14 (18), 16 (8), 18 (17), 19 (2), 20 (12), 25 (17), 26 (10) and 30 (16) were all *P. falciparum* infections in which there was a difficulty diagnosing the correct species (figures in brackets indicate number of incorrect species). All of these cases however had higher parasite densities being in the 6-49 or >50 cell categories. Confusing factors included stippling, Maurer's dots and EDTA changes.

Mixed infections

Case 39 showed a mixed infection of *P. falciparum* and *P. vivax* and was identified correctly by five participants. However, one participant identified *P. falciparum* and *P. ovale* infection showing the similarities in their morphology, and 18 participants identified *P. falciparum* infection alone. Two other participants identified *P. ovale* and *P. vivax* in isolation.

Comparison of images used in the initial assessment of the International group

Thick and thin films used

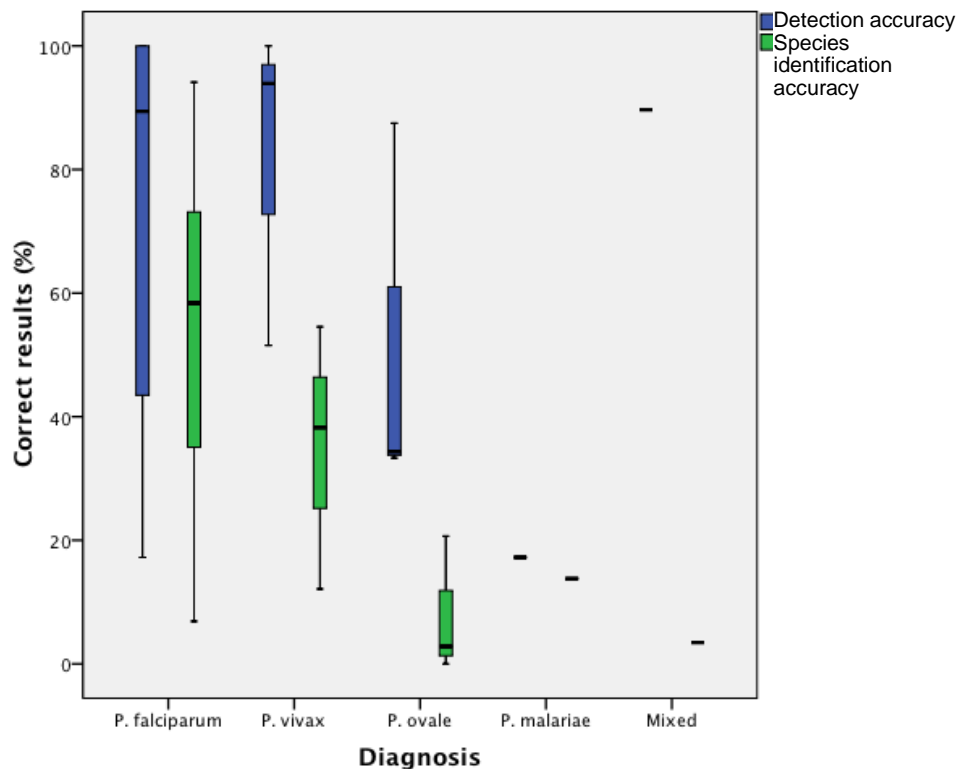
There were seven thick films and 33 thin films used in the International group. The detection accuracy for thick films was 68.5%(±31.5) and for thin films 76.4%(±28.9). The species identification accuracy for thick films was 38%(±30.1) and 44.7%(±28.2) for thin films. The differences between the thick and thin film were not significant (detection accuracy $p=0.276$) (species identification accuracy $p=0.581$).

Species

There was only one mixed species case, which was excluded from the analysis. The results for *P. malariae* were also obtained from a single case.

Figure 5.3 shows the comparison of detection accuracy and species identification accuracy for the different species and different slide preparations. The species identification accuracy for *P. falciparum* is higher than other species, with *P. ovale* specimens showing the lowest species identification accuracy at 6% (±6.5).

Figure 5.3: International Group: Comparison of the detection accuracy and species identification accuracy for the individual species in the initial assessment



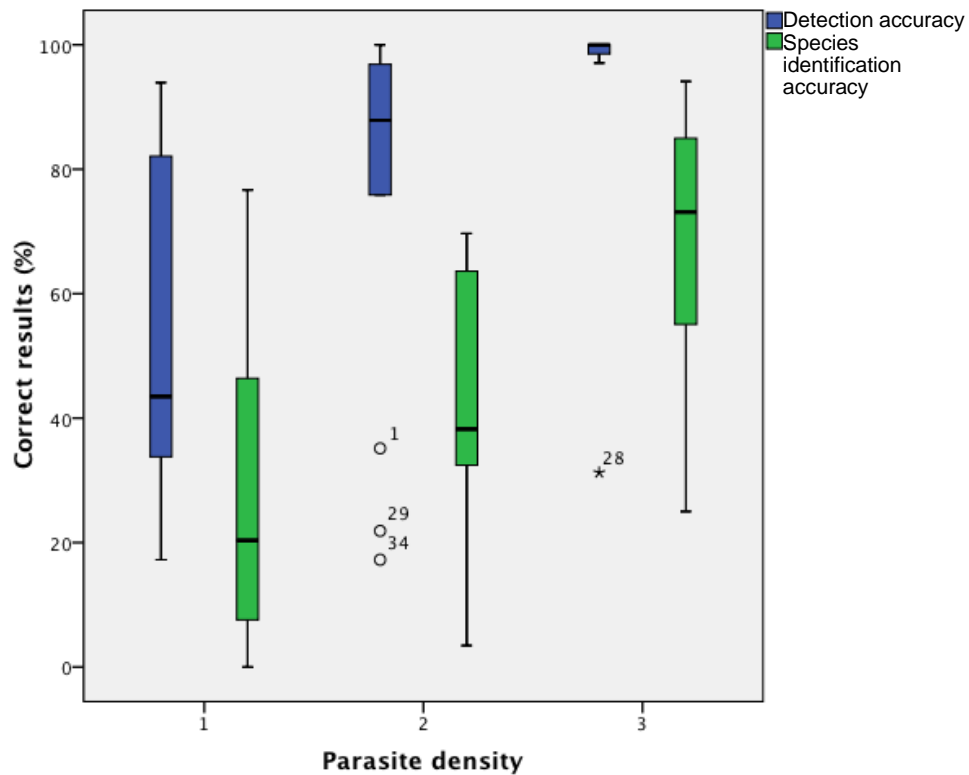
Negative samples had a detection accuracy of 90.4% (± 5.3), giving them the highest detection accuracy of all the cases used in the study, despite the presence of artefacts on many of these specimens.

There was a significant difference between the species identification accuracy ($p=0.010$) between the different species. There was no significant difference of the detection accuracy ($p=0.227$) between the different species.

Parasite density of specimens examined by the international group

The detection accuracy increased as the parasite density of specimens used in the international group increased (figure 5.4). There was a significant difference between the detection accuracy and the parasite density ($p=0.004$). There was a significant difference between the species identification accuracy and the parasite density ($p=0.012$).

Figure 5.4: International group: Comparison of detection accuracy and species identification accuracy for the rank of the parasite density in the initial assessment

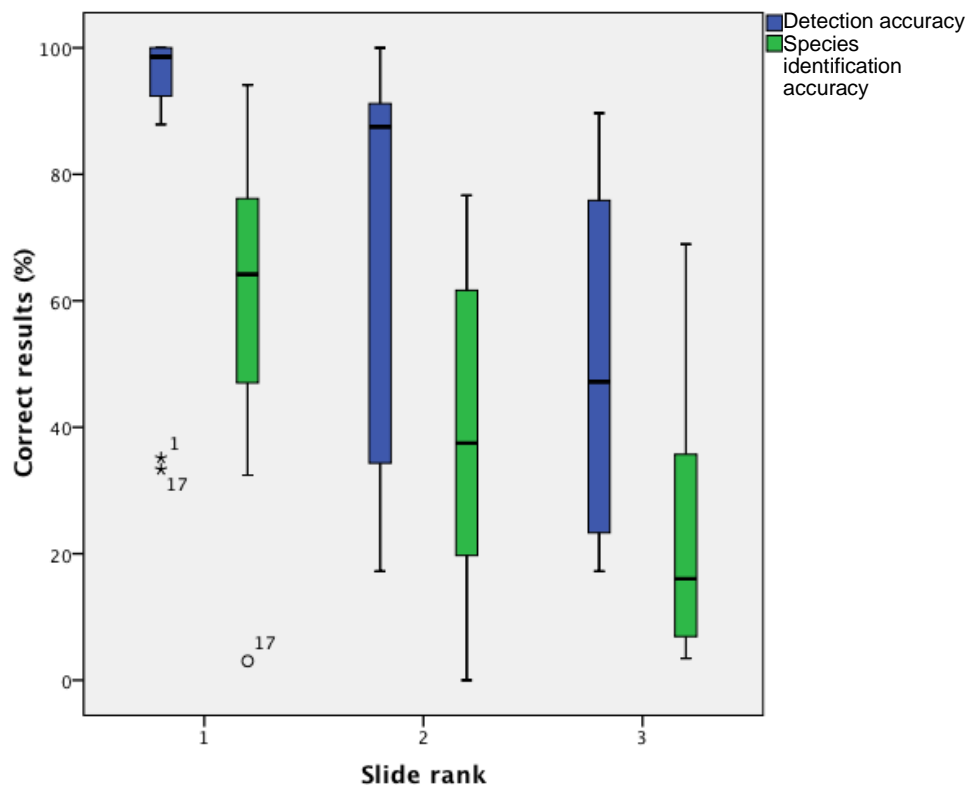


Circles indicate outliers, stars indicate extreme outliers

Overall ranking of the microscopic image

There was a significant difference between both the detection accuracy ($p=0.010$), and the species identification accuracy ($p=0.033$) and the rank of the microscopic image (figure 5.5).

Figure 5.5: International group: Comparison of detection accuracy and species identification accuracy for rank of the microscopic image in the initial assessment

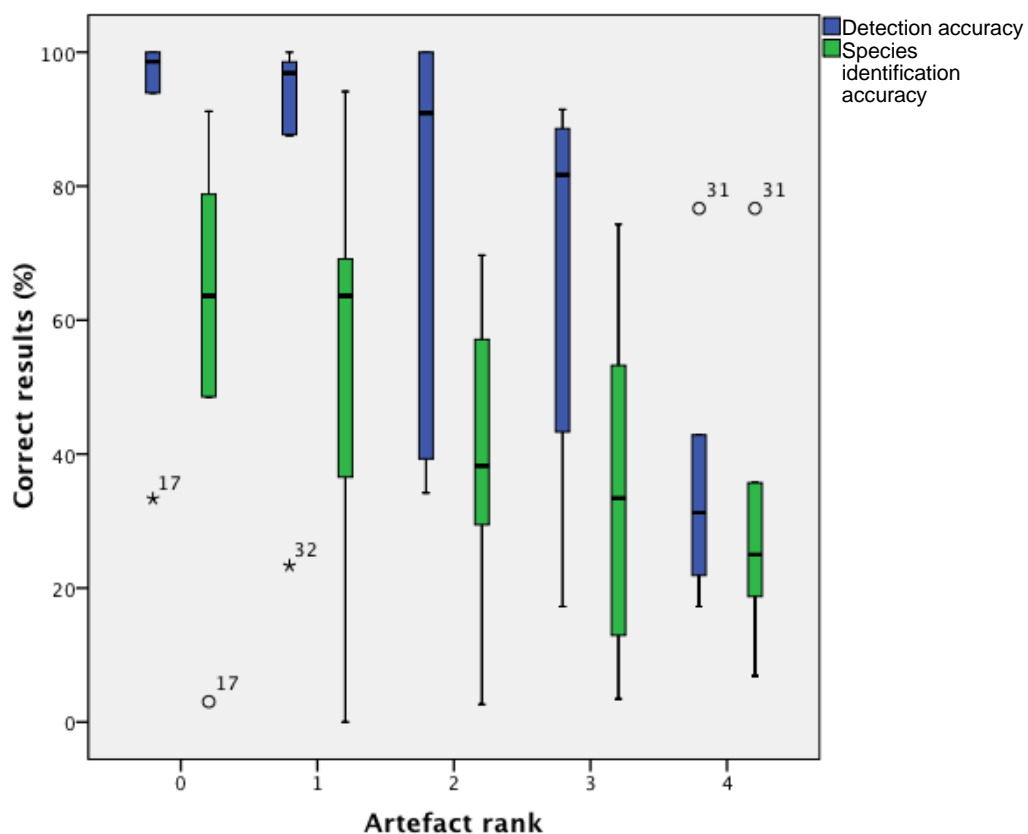


Circles indicate outliers, stars indicate extreme outliers

Effect of the presence of artefacts on the specimens

The presence of artefacts showed a decreasing detection accuracy and species identification accuracy when more artefacts were present. There was a significant difference in the detection accuracy ($p=0.026$), however, the species identification accuracy had not reached significance ($p=0.453$) (figure 5.6).

Figure 5.6: International group: Comparison of detection accuracy and species identification accuracy for the artefact rank in the initial assessment



Circles indicate outliers, stars indicate extreme outliers

Comparison of staff undertaking malarial diagnosis by microscopy in the International group

Table 5.5: Results from participants in initial assessment stage (n=40) for the International group

Location	Individual results	Definitive diagnosis			Detection accuracy	Species identification accuracy
		Positive	Negative	Total		
Kenya	Positive	139	1	140	86.7	67.6
	Negative	23	33	56		
	Total	162	34	196		
Hong Kong	Positive	24	0	24	96.7	84.0
	Negative	1	5	6		
	Total	25	5	30		
Ibadan 1	Positive	65	0	65	71.7	41.4
	Negative	34	21	55		
	Total	99	21	120		
Ibadan 2	Positive	62	9	71	61.7	21.2
	Negative	37	12	49		
	Total	99	21	120		
India	Positive	14	1	15	84.2	75.0
	Negative	2	2	4		
	Total	16	3	19		
Kano	Positive	14	0	14	76.2	47.4
	Negative	5	2	7		
	Total	19	2	21		
Lebanon	Positive	123	8	131	77.1	56.4
	Negative	38	29	67		
	Total	162	37	198		
Lagos 1	Positive	100	0	100	70.9	34.1
	Negative	50	32	82		
	Total	150	32	182		
Lagos 2	Positive	111	0	111	73.0	31.5
	Negative	54	35	89		
	Total	165	35	200		
Lagos 3	Positive	77	0	77	65.6	30.3
	Negative	55	28	83		
	Total	132	28	160		
Mean					76.4	48.9

Table 5.5 shows that the detection accuracy for laboratory staff participating in the international group was 76.4% (± 10.4), with a species identification accuracy of 48.9% (± 21.1). The majority of participants completed all 40 cases, with most others contributing a significant part. The results of any participant that completed less than ten cases were excluded.

Parasite detection

The overall detection accuracy was high, with eight participants reaching the correct diagnosis in more than 80% of cases, the highest rate being 97.5%. The majority of participants have a detection accuracy of between 70 and 80%. There were five participants with a detection accuracy of 60% or less, the lowest being 47.5%, an individual who had been working in the laboratory for 1-4 years.

Parasite speciation

The species identification accuracy was lower, with the best participant detecting this in 81.8% of cases. There were four participants that determined the correct species in more than 70% of cases. Twenty-four participants determined the correct species in less than 50% of cases, with the lowest being 9.1%, a participant with 5-9 years experience. One participant correctly identified the presence of parasites in 80.0% of cases, but only determined the species in 15.2% of these.

Experience of the laboratory staff

On grouping of the laboratory staff by experience there was a positive trend between the detection accuracy and individual experience. However, this did not reach significance ($p=0.104$). There was a significant difference for the species identification accuracy and the experience of the individual ($p=0.009$).

Training of the laboratory staff

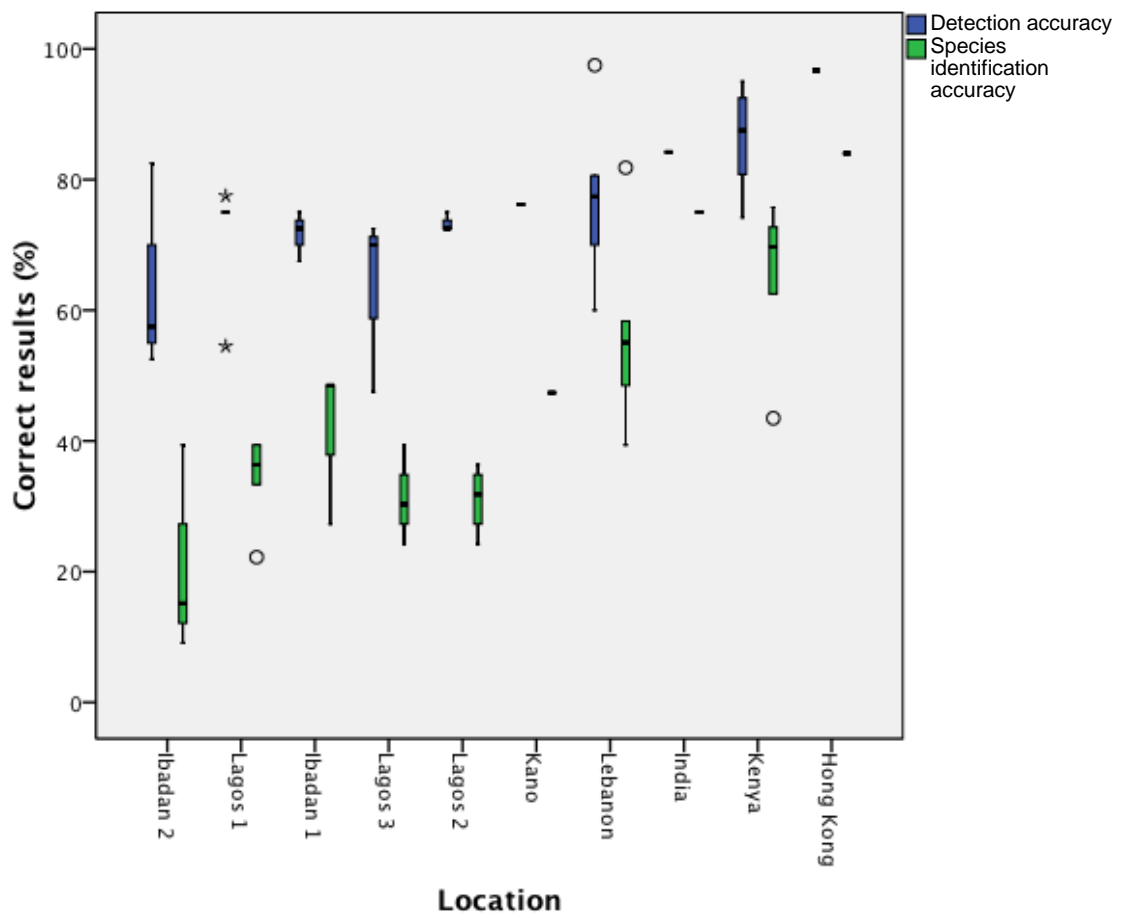
The time that had elapsed since laboratory workers had last received training on the diagnosis of malaria, had a moderate or no effect on the outcome of the diagnosis. There was no significant difference in the detection accuracy

($p=0.667$) and the species identification accuracy ($p=0.586$) in comparison to the time training was last received.

Geographical location of the participants

The locations of the participants were analysed to determine effects on the detection accuracy of diagnosis (Figure 5.7). Initial analysis by participant location, demonstrates that the laboratories that were involved in external quality assurance (EQA) schemes appeared to have higher detection accuracies and species identification accuracies. These EQA laboratories were Lebanon, India, Kenya and Hong Kong, with Kano in Nigeria in the process of implementing a training programme. There was a significant difference in the species identification accuracy when the participant location was considered ($p=0.006$). The detection accuracy however had not reached significance when compared with the location ($p=0.094$).

Figure 5.7: International group: The relationship between location and the results for detection and species identification accuracy in the initial assessment



Circles indicate outliers, stars indicate extreme outliers

5.4.2 Initial assessment results: UK group

In the initial assessment all of the UK participants were provided with the same 40 images as the International group and they were given the opportunity to provide a diagnosis for each case

The results from the individual cases are shown in table 5.6.

Table 5.6: The detection of parasites in the initial assessment stage cases (n=40) for the UK participants group.

Definitive diagnosis	n (=40)	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)
Negative	7	90.1	18.2	N/A	N/A
<i>P. falciparum</i>	24	92.6	50.0	72.5	64.3
<i>P. vivax</i>	3	71.9	84.4	40.6	56.3
<i>P. ovale</i>	4	90.9	29.4	41.0	62.3
<i>P. malariae</i>	1	88.9	N/A	29.6	N/A
Mixed infection	1	100.0	N/A	37.0	N/A

N/A not applicable

Overall, the diagnosis of malaria for all UK participants across all 40 cases gave a detection accuracy of 90.5% (± 16.2). However, there was not one case that was correctly diagnosed by all participants in terms of determining the presence of parasites and the correct species. There were 16 cases in which all participants correctly identified the presence or absence of parasites, one of these specimens was a negative case, and the correct species was determined by all participants in two of the 15 cases. The species identification accuracy was also low at 63.4% (± 23.7).

Table 5.7 demonstrates the performance on the individual cases for the UK group in the initial assessment.

Table 5.7: The performance on the individual cases (n=40) in the initial assessment by participants in the UK group

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
1	<i>P. falciparum</i>	97.1	91.2	Yes	2	3	1
2	<i>P. ovale</i>	70.6	11.8		1	2	2
3	Negative	91.2			-	4	2
4	<i>P. falciparum</i>	97.1	73.5		1	3	2
5	Negative	81.8			-	4	2
6	<i>P. falciparum</i>	100.0	93.9		3	0	1
7	Negative	93.8			-	3	1
8	<i>P. vivax</i>	100.0	59.4		2	2	2
9	<i>P. falciparum</i>	100.0	65.6		3	1	1
10	<i>P. vivax</i>	15.6	3.1	Yes	1	3	3

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
11	<i>P. falciparum</i>	100.0	90.6		2	1	1
12	<i>P. falciparum</i>	100.0	75.0		3	0	1
13	<i>P. falciparum</i>	100.0	59.4		3	0	1
14	<i>P. falciparum</i>	100.0	87.5		3	2	1
15	<i>P. falciparum</i>	84.4	78.1	Yes	2	2	2
16	<i>P. falciparum</i>	100.0	71.9		2	1	1
17	<i>P. ovale</i>	96.9	50.0		1	0	1
18	<i>P. falciparum</i>	100.0	71.9		2	0	1
19	<i>P. falciparum</i>	100.0	100.0		3	1	1
20	<i>P. falciparum</i>	100.0	75.0		3	1	1
21	<i>P. falciparum</i>	90.6	62.5		1	2	2

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
22	<i>P. vivax</i>	100.0	59.4		2	0	2
23	Negative	87.5			-	1	2
24	<i>P. ovale</i>	100.0	28.1		1	1	2
25	<i>P. falciparum</i>	100.0	50.0		2	3	2
26	<i>P. falciparum</i>	100.0	100.0		2	2	2
27	Negative	83.9		Yes	-	0	2
28	<i>P. falciparum</i>	93.8	65.6		3	4	2
29	<i>P. falciparum</i>	75.0	56.3		2	4	2
30	<i>P. falciparum</i>	96.9	62.5		2	3	2
31	<i>P. falciparum</i>	96.6	86.2		1	4	3
32	<i>P. falciparum</i>	75.0	67.9		1	1	2

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
33	Negative	100.0			-	4	2
34	<i>P. falciparum</i>	50.0	35.7	Yes	2	4	3
35	Negative	92.3		Yes	-	4	3
36	<i>P. falciparum</i>	77.8	48.2	Yes	2	3	3
37	<i>P. falciparum</i>	88.9	70.4		1	4	3
38	<i>P. ovale</i>	96.3	74.1		1	2	2
39	<i>P. falciparum</i> and <i>P. ovale</i>	100.0	37.0		2	3	3
40	<i>P. malariae</i>	88.9	29.6		1	3	2
Average		90.5	63.4				

False negatives

Low parasite density

The presence of parasites at a low density caused some false negative results. Ten out of the 12 cases presented at low parasite density showed some false negative results. Case 2 had ten false negative results, this was a *P. ovale* case with one parasite present on the image, showing gametocytes. Other false negative results were reported for cases 4 (1), 17 (1), 21 (3), 31 (1), 32 (5), 37 (3), 38 (1) and 40 (3) (brackets indicate number of false negatives per case).

Thick films

The greatest difficulty in diagnosis, in this group, was the detection and speciation of parasites on the thick film. The detection accuracy of the thick film was 71.6% (± 29.0) for the seven thick films used. In case 10, 27 participants failed to identify the presence of parasites. False negative results were also reported in cases 1 (1), 15 (5), 34 (14) and 36 (6) (brackets indicate number of false negatives per case).

Artefacts

There were five cases with the highest category of artefacts present, all of which had some false negative results. Case 28 had two false negative results, some of the parasites in this film are faint and could be confused with stain deposit. The diagnosis on case 29 was complicated by the presence of Chronic Granulocytic Leukaemia, with eight participants missing the presence of *P. falciparum*.

False positives

Thick films

There were two negative thick films used in the initial assessment for the UK group, one of which had false positive results. Case 27 was determined to be positive by five participants, presumably due to confusion with artefacts.

Artefacts

False positive results were seen for six out of the seven negative cases used. Case 3 had three false positive results possibly because of stain deposit, case 7 had two false positive results probably due to basophilic stippling, case 35 a thick film had two false positive results.

Incorrect species

Low parasite density

Twelve low parasite density cases were used in the initial assessment, all of which caused problems with parasite identification, possibly due to the lack of cells present to allow identification. There were six *P. falciparum* cases that caused difficulties, cases 4 (8), 6 (2), 21 (9), 31 (3), 32 (4) and 37 (5). There were two *P. vivax* low parasite density infections (cases 10 (4) and 22 (6)) which had incorrect species identified (brackets indicate the number of incorrect species identified).

All four *P. ovale* cases used in the initial assessment (cases 2 (20), 17 (16), 24 (20) and 38 (6)) were at low parasite density and caused difficulties in diagnosis made. As the *P. ovale* infected cells have a similar appearance to *P. vivax* and receive the same treatment any confusion between these species was regarded as a minor error.

Case 40, a *P. malariae* infection had only two parasites present, 16 individuals incorrectly diagnosed the species.

Thick films

The species is not normally determined on the thick film and is not recommended practice in the UK. There were five positive thick films cases 1 (2), 10 (4), 15 (2), 34 (4) and 37 (5) (brackets indicate the number of incorrect species identified).

Artefact

Artefacts cannot only cause confusion in identifying whether parasites are present, but also can look like different species, in cases 25 (16), 28 (9) and 29 (6) (brackets indicate the number of incorrect species identified).

Cell inclusions

The majority of cases that caused problems in speciation were affected by EDTA, increasing the number of Accole forms and Maurer's clefts present. Cases 4 (8), 6 (2), 9 (11), 12 (8), 13 (13), 18 (9), and 20 (8), all had diagnoses of *P. vivax* and *P. ovale* made due to the presence of Maurer's clefts being confused with stippling. Cases 11 (3), 14 (4), 16 (9), 20 (8) and 30 (11) also had a diagnosis of *P. malariae* as well as *P. vivax* and *P. ovale* (brackets indicate the number of incorrect species identified).

P. ovale and *P. vivax* cases

The main difficulty in the UK group was determining the species in *P. ovale* and *P. vivax* infections as they have a very similar appearance. However, both species have the same treatment, therefore a sub category of treatment species identification accuracy, was analysed. The overall treatment species identification accuracy was increased to 70.8% (± 21.2) in comparison to the species identification accuracy. The treatment species identification accuracy for *P. ovale* was 80% (± 17.9), with a species identification accuracy of 41.0% (± 27.1). The same was seen for *P. vivax* with the species identification accuracy increasing from 40.6% (± 32.5) to 61.5% (± 48.4). The increase in the treatment species accuracy indicates that most species misidentification were for the alternative species. Case 2 for example, a *P. ovale* case, was only correctly diagnosed by four participants, 16 participants diagnosed this case as *P. vivax* this would increase the species identification accuracy from 11.8% to 58.8%.

Mixed infections

Case 39 showed a mixed infection of *P. ovale* and *P. falciparum*. Ten individuals identified that both species were present, nine identifying *P. falciparum* alone. Seven individuals mistook *P. ovale* for *P. vivax* and one identified only *P. vivax*.

Comparison of cases used in the initial assessment for the UK group

Thick and thin films

There were seven thick films and 33 thin films. The detection accuracy for thick films was 71.6% (± 29.0) and 94.6% (± 8.2) for thin films. The species identification accuracy of thick films was 51.3% (± 34.9) and for thin films 65.5% (± 21.3). There was a significance difference in detection accuracy ($p=0.003$) between the thick and thin films. However, the species identification accuracy for the thick and thin films did not reach significance ($p=0.421$).

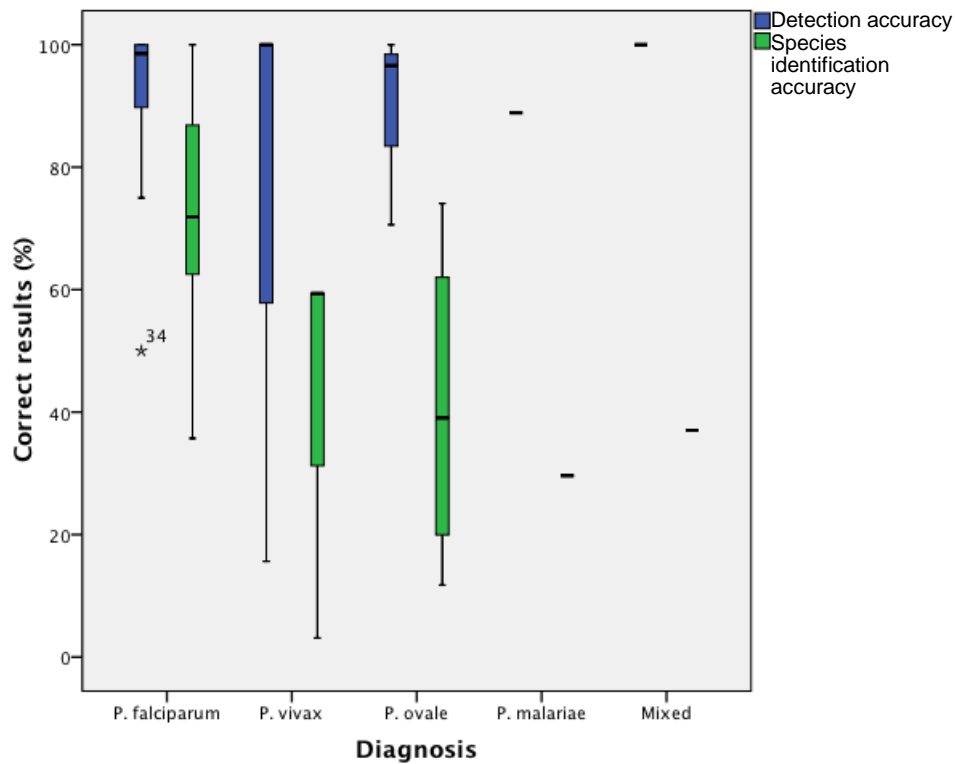
Species identification

There was only one mixed species case, due to the small number of results, this case was excluded from the analysis. The results for *P. malariae* were obtained from a single case.

Figure 5.8 shows the comparison of detection accuracy and species identification accuracy for the different species and different slide preparations. The species identification accuracy for *P. falciparum* was higher than the other species ($72.5 \pm 16.6\%$).

There was a significant difference in the species identification accuracy ($p=0.025$) between the different malaria species. However, the detection accuracy did not reach significance ($p=0.494$) when compared to the different malaria species.

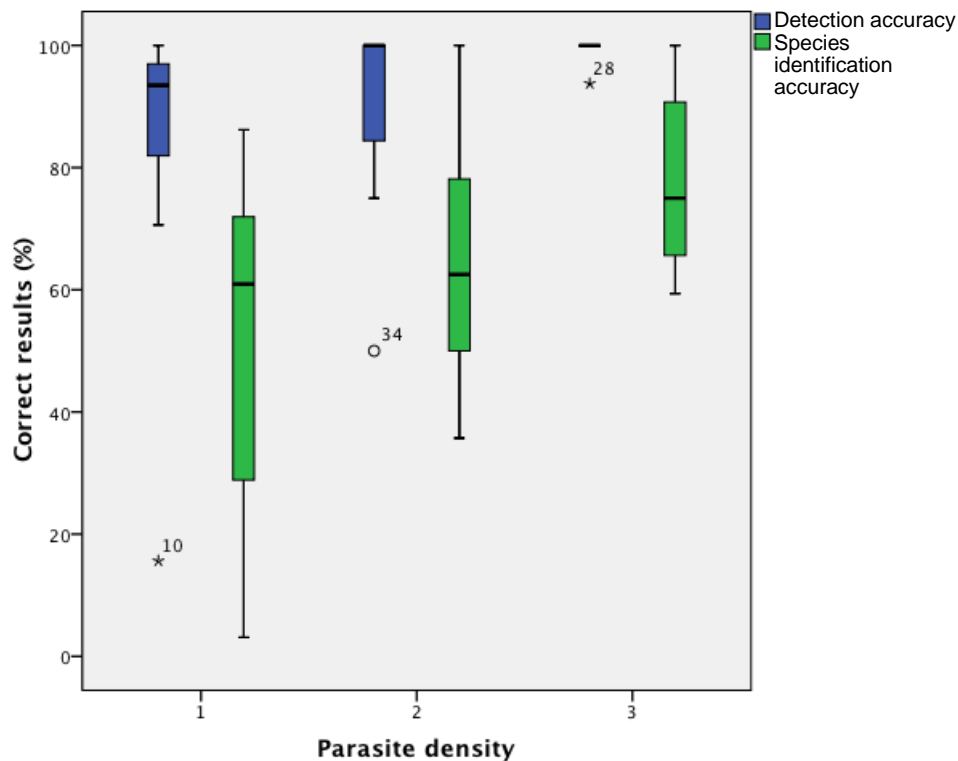
Figure 5.8: UK group: Comparison of detection accuracy and species identification accuracy on cases of different species in the initial assessment



Stars indicate extreme outliers

Parasite density of case images

Figure 5.9: UK group: Comparison of detection and species identification accuracy and the rank of the parasite density in the initial assessment



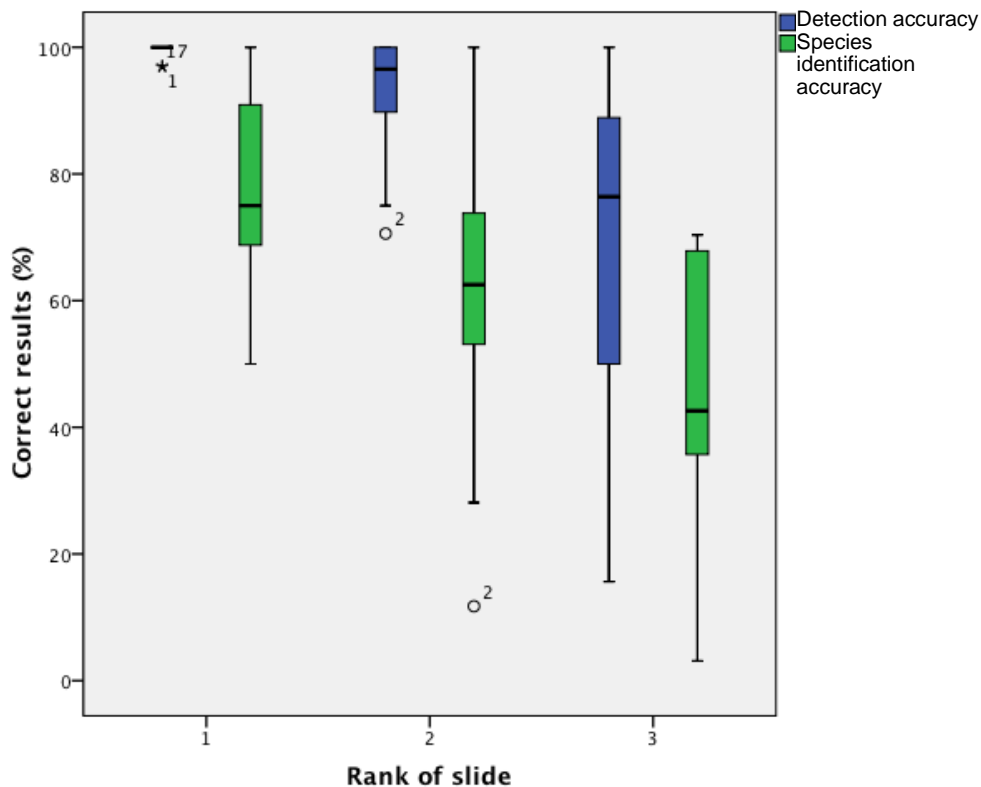
Circles indicate outliers, stars indicate extreme outliers

There was a significance difference in the detection accuracy ($p=0.017$) when the parasite density of the specimen increased (figure 5.9). However, the species identification accuracy only approached significance ($p=0.064$).

Overall ranking of the microscopic image

The rank of the microscopic image demonstrated a highly significant difference for the detection accuracy ($p=0.001$), and a significant difference for the species identification accuracy ($p=0.010$) (figure 5.10). The detection accuracy for rank 1, the easiest group was close to 100%, this falls to 73% for rank 3, when the case was deemed to be the most difficult.

Figure 5.10: UK group: Comparison of detection and species identification accuracy and the rank of the microscopic image in the initial assessment



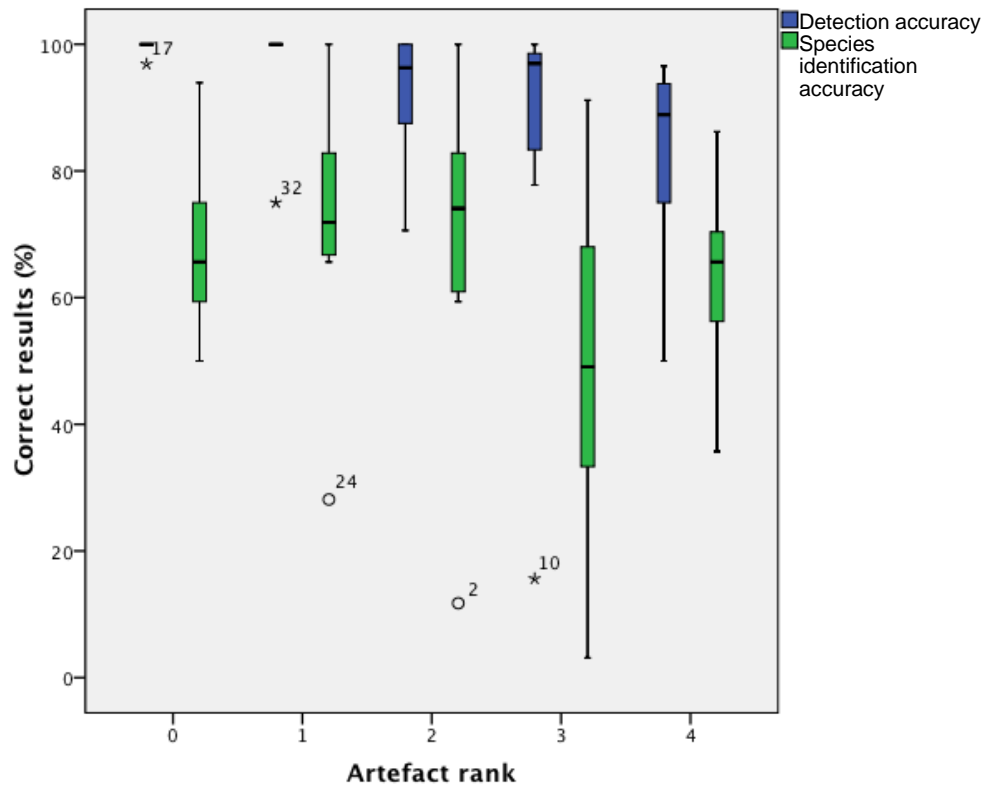
Circles indicate outliers, stars indicate extreme outliers

Presence of artefact

In the presence of artefacts a decreasing detection accuracy was found but no trend in species identification accuracy was found when more artefacts were present (figure 5.11). The results for both the detection accuracy

($p=0.093$) and species identification accuracy ($p=0.382$) were not significantly different when more artefacts were present.

Figure 5.11: UK group: Comparison of the detection and species identification accuracy and the artefact rank in the initial assessment



Circles indicate outliers, stars indicate extreme outliers

Comparison of staff undertaking malarial diagnosis by microscopy in the UK group

Table 5.8: Results from individual participants for the initial assessment stage cases (n=40) in the UK group

Location	Individual results	Definitive diagnosis			Detection accuracy (%)	Species identification accuracy (%)
		Positive	Negative	Total		
1	Positive	146	3	149	92.1	75.6
	Negative	12	30	42		
	Total	158	33	191		
2	Positive	215	3	218	91.9	72.1
	Negative	22	47	69		
	Total	237	50	287		
3	Positive	121	3	124	91.3	70.5
	Negative	11	25	36		
	Total	132	28	160		
4	Positive	59	1	60	90.0	51.5
	Negative	7	13	20		
	Total	66	14	80		
5	Positive	86	3	89	87.5	54.6
	Negative	13	18	31		
	Total	99	21	120		
6	Positive	90	2	92	90.8	48.5
	Negative	9	19	28		
	Total	99	21	120		
7	Positive	30	0	30	92.5	45.5
	Negative	3	7	10		
	Total	33	7	40		
8	Positive	154	5	159	91.9	64.9
	Negative	11	28	39		
	Total	165	33	198		
9	Positive	28	2	30	82.5	42.4
	Negative	5	5	10		
	Total	33	7	40		
Mean					90.1	58.4

There were 24 participants that completed all 40 cases in the initial assessment. Another seven completed more than 30 of the cases. One participant was excluded from the analysis due to the small number of cases completed.

Table 5.8 describes the results for the UK participants over the 40 cases or those that they attempted. The detection accuracy for laboratory staff participating was 90.1% (± 3.2), with a species identification accuracy of 58.4% (± 12.5).

Parasite detection

One participant detected the presence or absence of parasites correctly in the 13 cases they completed. Twenty-two participants detected the parasites present in more than 90% of cases. Two participants achieved a detection accuracy of 97.5%. The lowest detection accuracy was 77.5%, an individual with less than two years experience.

Parasite speciation

A clear difference can be seen between detecting the presence of parasites and determining the correct species. The highest species identification accuracy was 87.9%, with the lowest at 39.4%.

Experience of the laboratory staff

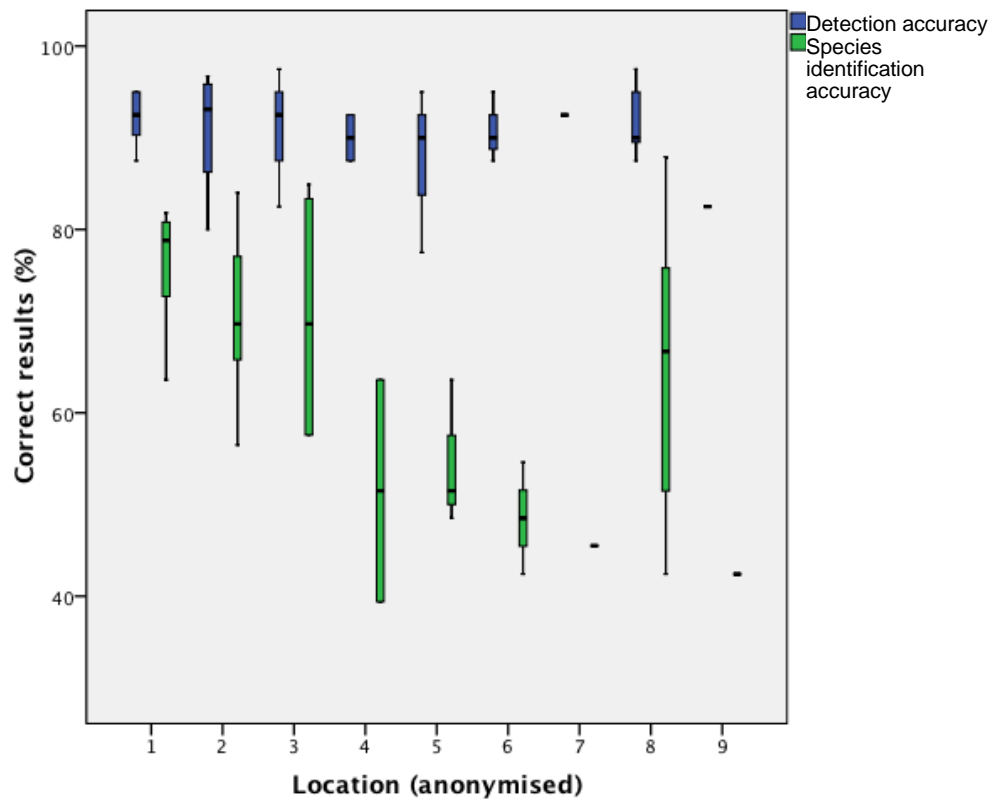
The participants were divided into two groups depending upon their experience. Group one refers to those with less than two years experience or newly registered Biomedical Scientists. Group two is the individuals with more than five years experience, varied from five years up to more than 20 years. The detection accuracy for group one, those with less than two years experience was 90.0% (± 5.8) and for group two was 92.1% (± 5.2), this difference was not significant ($p=0.171$). The species detection accuracy for group one was 55.8% and group two was 68.8%, this difference was significant ($p=0.009$).

Location of the laboratory staff

There was no significant difference in detection accuracy results ($p=0.918$) between the different hospitals in which the participants were based. There

was no significant difference in the species identification accuracy ($p=0.053$) for the participants location (figure 5.12).

Figure 5.12: UK group: The relationship between the location and the results for detection and species identification accuracy in the initial assessment



5.4.3 Comparison of UK and International results in the initial assessment

The results of the initial assessment for both the International and UK groups were compared. The differences between the groups were assessed to determine where differences were and how they may have occurred.

There were 18 individuals in the international group that completed all 80 cases and 13 in the UK group. Table 5.9 shows the initial assessment results from the International group and table 5.10 from the UK group. The tables give details of every response to the image, not just for the 40 cases but also for all the individuals completing.

Table 5.9: Results of the 18 international participants for the 40 cases in the initial assessment

	Participant responses						
<i>Definitive diagnosis</i>	<i>Total responses</i>	<i>Negative</i>	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>	<i>Mixed</i>
<i>Negative</i>	126	115	3	4	2	2	0
<i>P. falciparum</i>	432	132	184	69	13	34	0
<i>P. vivax</i>	54	8	28	11	3	4	0
<i>P. ovale</i>	72	53	2	8	2	7	0
<i>P. malariae</i>	18	17	0	0	0	1	0
<i>Mixed</i>	18	3	14	0	1	0	0
<i>Total</i>	720	328	231	92	21	48	0

The total number of responses for each species and negatives are indicated

Table 5.10: Results of the 13 UK participants for the 40 cases in the initial assessment

	Participant responses						
<i>Definitive diagnosis</i>	<i>Total responses</i>	<i>Negative</i>	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>	<i>Mixed</i>
<i>Negative</i>	91	84	2	1	1	3	0
<i>P. falciparum</i>	312	16	249	17	13	15	2
<i>P. vivax</i>	39	11	3	17	7	1	0
<i>P. ovale</i>	52	2	2	23	23	2	0
<i>P. malariae</i>	13	2	2	0	5	4	0
<i>Mixed</i>	13	0	4	0	0	0	9
<i>Total</i>	520	115	262	58	49	25	11

The total number of responses for each species and negatives are indicated

Although the numbers of individuals between the two groups are different, the percentage of results can be compared as well as the false positive, true positive, false negative and true negative results. Table 5.6 shows that for the international group there were 11 (8.7%) false positive results, with table 5.7 showing the UK had seven (7.7%). There were 381 (64.1%) true positive results for the international group and 398 (92.8%) for the UK group. There were 115 (91.3%) true negative results in the international group and 84 (92.3%) for the UK. There were 213 (35.9%) false negative results from the international group and 31 (7.2%) from the UK group.

The international participants determined the incorrect species in 183 (30.8%) instances, for the UK group this was 96 (22.4%). Speciation for the UK group showed difficulty in determining the difference between *P. ovale* and *P. vivax*, if these differences are excluded the incorrect species was determined in 66 instances. The international group had difficulty determining

the species in a number of cases, with many species being identified as *P. falciparum*, but also *P. falciparum* cases being identified as different species.

Differing participant performances were found on the thick and thin films. There were seven thick films in the initial assessment, with 24 of the 31 false negative instances for the UK group being on the thick film. In the international group, there were 47 false negative instances and 16 incorrect species. The majority of these instances in both groups were from the same case, case 10 a *P. vivax* thick film that had only a few parasites present.

Table 5.11: Initial assessment, percentage detection accuracy and species identification accuracy for both the UK and International group

	International group		UK group	
	Detection accuracy (%)	Species identification accuracy (%)	Detection accuracy (%)	Species identification accuracy (%)
All	68.9	33.4	92.3	69.9
Thick	61.1	30.0	71.4	52.3
Thin	70.5	33.9	96.7	73.1
<i>P. falciparum</i>	69.4	42.4	94.7	79.6
<i>P. vivax</i>	85.2	22.2	71.8	43.6
<i>P. ovale</i>	26.4	2.8	96.2	44.2
<i>P. malariae</i>	5.6	5.6	84.6	30.8
Negative	91.3	N/A	92.3	N/A

N/A= not applicable

Table 5.11 compares the results of both groups in the initial assessment, the detection accuracy was greater for the UK group on all cases except for *P. vivax* cases. There was a highly significant differences between the detection accuracy ($p=0.001$) and the species identification accuracy ($p<0.001$) between the UK and International group.

5.5 Intervention training stage

5.5.1 International group

The International group were given access to the training stage over a four-month period. The training was released in two stages, with the thin film training be provided initially and then followed by the thick film training. The training programme can be viewed on the appendix 1.8.

5.5.2 UK group

The training was provided to the UK group over a six-week period. The training was delivered all at the same time.

5.6 Final assessment

5.6.1 International group

Of the 42 participants, initially recruited 26 participants took part in the final assessment stage. Twenty-one of these participants completed all 40 cases in the final assessment. One participant only completed three cases and was therefore excluded from the analysis based on individual participant results.

The results from the individual cases are shown in table 5.12.

Table 5.12: The detection of parasites in the final assessment stage cases (n=40) for the International participants group.

Definitive diagnosis	n (=40)	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)
Negative	7	95.2	20.8	NA	NA
<i>P. falciparum</i>	24	74.6	78.0	51.8	78.0
<i>P. vivax</i>	3	36.4	90.9	7.3	21.7
<i>P. ovale</i>	3	23.3	25.7	13.0	26.1
<i>P. malariae</i>	2	21.6	6.8	8.7	9.1
Mixed infection	1	90.9	NA	4.6	NA

Diagnosis of malaria for all participants across all 40 cases gave a detection accuracy of 69.1% (± 35.5). There were five cases, in which all participants correctly identified the presence or absence of parasites. There were not any cases in which the correct species was determined by all participants. The species identification accuracy was low at 40.2% (± 28.8). Of the cases that were positive, only one case had the correct species identified by all participants that determined that parasites were present, this was case 62.

Table 5.13: Performance on the individual cases in the final assessment by the International group

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
41	Negative	79.2			-	3	2
42	<i>P. falciparum</i>	100.0	83.3		3	1	1
43	<i>P. falciparum</i>	100.0	58.3		1	4	2
44	<i>P. falciparum</i>	82.6	78.3	Yes	2	4	2
45	<i>P. falciparum</i>	100.0	79.2		3	0	1
46	<i>P. ovale</i>	26.1	13.0		1	1	3
47	Negative	100.0		Yes	-	2	2
48	<i>P. falciparum</i>	100.0	65.2		3	1	1
49	<i>P. falciparum</i>	17.4	17.4	Yes	2	4	3
50	<i>P. falciparum</i>	34.8	30.4		2	2	2
51	<i>P. falciparum</i>	100.0	65.2		3	2	1

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
52	<i>P. falciparum</i>	100.0	65.2		3	1	1
53	Negative	95.7			-	2	1
54	<i>P. falciparum</i>	91.3	60.9		2	3	1
55	<i>P. falciparum</i>	95.7	43.5		2	0	1
56	Negative	91.3			-	2	1
57	<i>P. falciparum</i>	95.7	43.5		3	1	1
58	<i>P. falciparum</i>	100.0	82.6		2	3	1
59	<i>P. falciparum</i>	100.0	43.5		3	0	1
60	<i>P. falciparum</i>	95.7	34.8		3	0	1
61	<i>P. falciparum</i>	12.0	12.0		1	2	2
62	<i>P. falciparum</i>	44.0	44.0		1	3	2
63	<i>P. malariae</i>	25.0	8.3	Yes	1	3	2

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
64	Negative	100.0			-	3	2
65	<i>P. falciparum</i>	34.8	13.0		1	3	2
66	<i>P. ovale</i>	34.8	26.1		1	1	2
67	<i>P. falciparum</i>	39.1	34.8		2	1	2
68	Negative	100.0			-	2	2
69	<i>P. vivax</i>	95.7	21.7		2	2	2
70	<i>P. falciparum</i>	82.6	78.3		2	3	2
71	<i>P. vivax</i>	8.7	0.0	Yes	2	3	2
72	<i>P. falciparum</i>	63.6	31.8	Yes	2	3	3
73	<i>P. falciparum</i>	95.5	95.5		1	2	2
74	<i>P. malariae</i>	18.2	9.1		1	3	2
75	<i>P. ovale</i>	9.1	0.0		1	0	3

Case	Definitive diagnosis	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
76	<i>P. falciparum</i>	81.8	63.6		1	2	3
77	<i>P. falciparum</i> and <i>P. ovale</i>	90.9	4.6	Yes	2	4	3
78	Negative	100.0			-	3	2
79	<i>P. falciparum</i>	18.2	18.2		1	1	3
80	<i>P. vivax</i>	4.8	0.0	Yes	1	3	3
Average		69.1	40.2				

Table 5.13 demonstrates performance on the individual cases in the final assessment for the international group.

False negative results

There were 14 cases in which more than 14 participants failed to identify the presence of parasites. The false negative results were split into categories as follows:

Low parasite density

There were ten out of 13 low parasite density cases, which had false negative results. *P. ovale* thin films had a number of false negative results on cases 46 (17), 66 (15) and 75 (20) (number of false negatives in brackets). The same difficulty was presented on case 80 a thick film *P. vivax* case with 20 false negative results.

Case 63 a *P. malariae* thick film also had a high false negative rate, with 18 participants not identifying parasites were present. A *P. malariae* thin film case 74 also had 18 participants determining parasites were not present.

The remaining cases were all *P. falciparum* thin films all of which had a low parasite density. Cases 61 (22), 62 (14), 65 (15), 67 (14), 76 (4) and 79 (18) showed these false negative results.

Thick films

Seven positive thick films were used, however all of these were falsely determined to be negative by some participants. False negative results were seen on cases 44 (4), 49 (19), 71 (21), 72 (8) and 80 (20) (brackets indicate number of instances).

Artefacts

Cases 43, 44, 49 and 77 had the highest quantity of artefacts present. There was one participant in case 43, two on case 44, 19 on case 49 and two on case 77 who called the case negative. Case 44 was a thick film, so staining artefact was present and parasites were difficult to see in the background staining.

False positive results

There were three out of seven negative cases in which false positive results were identified by a small number of individuals. Case 41 was identified as positive by five individuals. Two individuals falsely classified case 56 as positive, with one individual determining case 53 to be positive.

Incorrect species

Determining the correct species seemed to be the most difficult task for the individuals, even when the presence of parasites had been correctly detected. There were seven cases in which more than ten individuals incorrectly determined the species present.

There were 24 *P. falciparum* cases in total, 19 of which were identified as a different species by one or more individuals. There were only two *P. malariae* cases used in the final assessment but difficulties in diagnosis were seen in both of these. Case 63 was a thick film, with four incorrect diagnoses and case 74, a thin film, with two participants determining the incorrect species. There were three *P. vivax* cases used in the final assessment all of which had incorrect species determined, cases 69 (17), 71 (2) and 80 (1) (brackets indicate the number of incorrect species identified).

Low parasite density

Case 76 had a low parasite density, there were four individuals that reported the wrong species was present.

Artefacts

Case 43 had very high levels of artefact present, the incorrect species was recorded by ten participants. Cases 54, 58, 67, 72 and 77 also had high numbers of artefacts present, difficulty in species determination was seen.

Thick films

Three out of the seven positive cases, cases 63, 72 and 77 caused difficulties in diagnosis. Case 77 showed a mixed infection of *P. falciparum* and *P. ovale*, nine participants only identified *P. falciparum* was present.

Cell inclusions

Cases 42 (4), 45 (5), 48 (8), 51 (8), 52 (7), 55 (12), 57 (12), 59 (13) and 60 (14) show high parasite density *P. falciparum* cases where the species was incorrectly identified (brackets indicate the number of incorrect species identified).

Comparison of cases used in the final assessment for the International group

Thick and thin films

There were eight thick films and 32 thin films in the final assessment. The detection accuracy for thick films was 49.1% (± 39.4) and 74.1% (± 33.3) for thin films. The species identification accuracy for thick films was 20.1% (± 28.0) and thin films 45.6% (± 27.0). There were significant differences in the detection accuracy ($p=0.039$) and the species identification accuracy ($p=0.021$) between the thick and thin films.

Species

Figure 5.13 demonstrates the comparison of the detection accuracy and species identification accuracy for the different species present. The lowest species identification accuracy shown is for that of *P. vivax* at 7.3% (± 12.6), influenced by one thick film case. Negative films show that the participants are able to correctly determine that parasites are not present in 95.2% (± 7.8) cases.

There was a significant difference between both the detection accuracy ($p=0.022$) and species identification accuracy ($p=0.003$) for the different species.

Parasite density of case images

Figure 5.14 demonstrates how the detection accuracy and species identification accuracy increases as the parasite density increases.

There was a highly significant difference between the detection accuracy and the parasite density ($p<0.001$). There was a significant difference between the species identification accuracy and the parasite density ($p=0.025$).

Figure 5.13: International Group: Comparison of the detection and species identification accuracy for the different species present in the final assessment

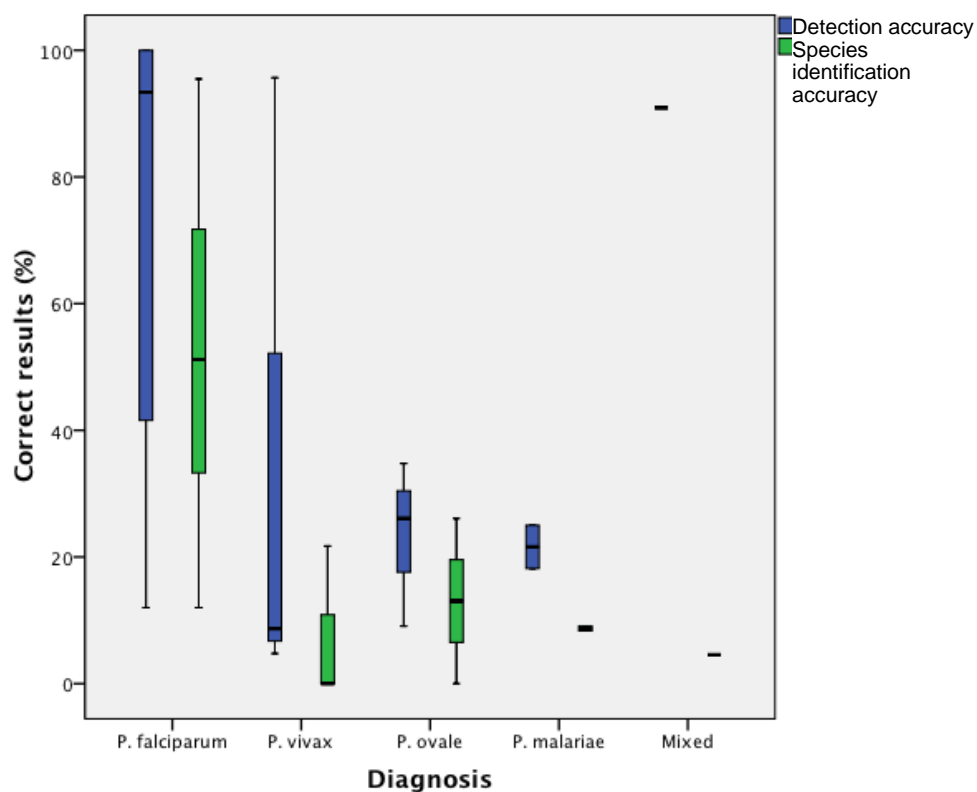
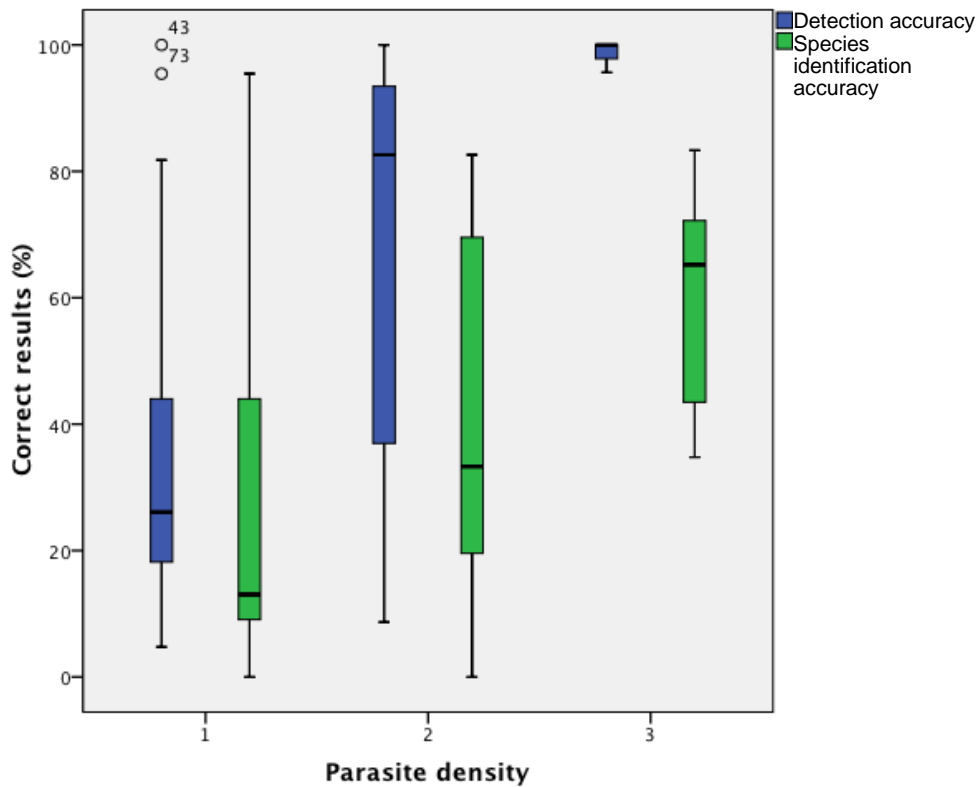


Figure 5.14: International group: Comparison of the detection and species identification accuracy for the parasite density rank in the final assessment

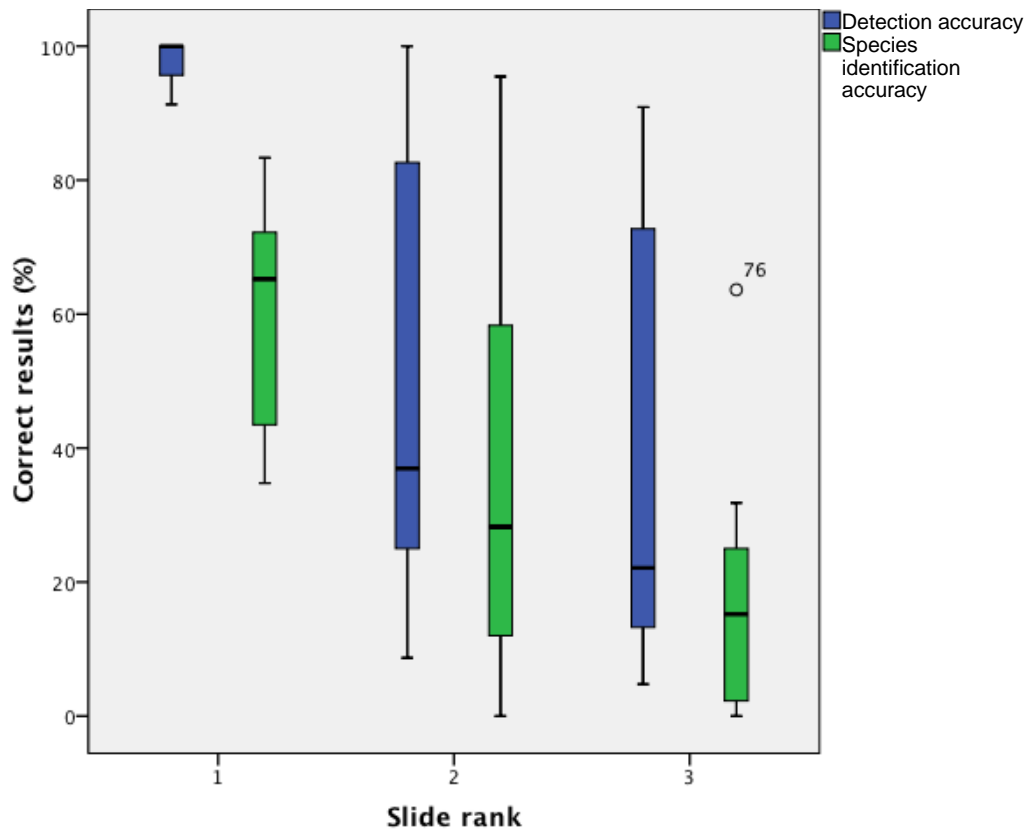


Circles indicate outliers,

Overall ranking of the microscopic image

Figure 5.15 demonstrates the results of each ranking category based on the difficulty of the microscopic image. There was a highly significant difference between the detection accuracy ($p < 0.001$) and the species identification accuracy ($p = 0.003$), when compared to the rank of the microscopic image.

Figure 5.15: International group: Comparison of detection and species identification accuracy with the rank of the microscopic image in the final assessment

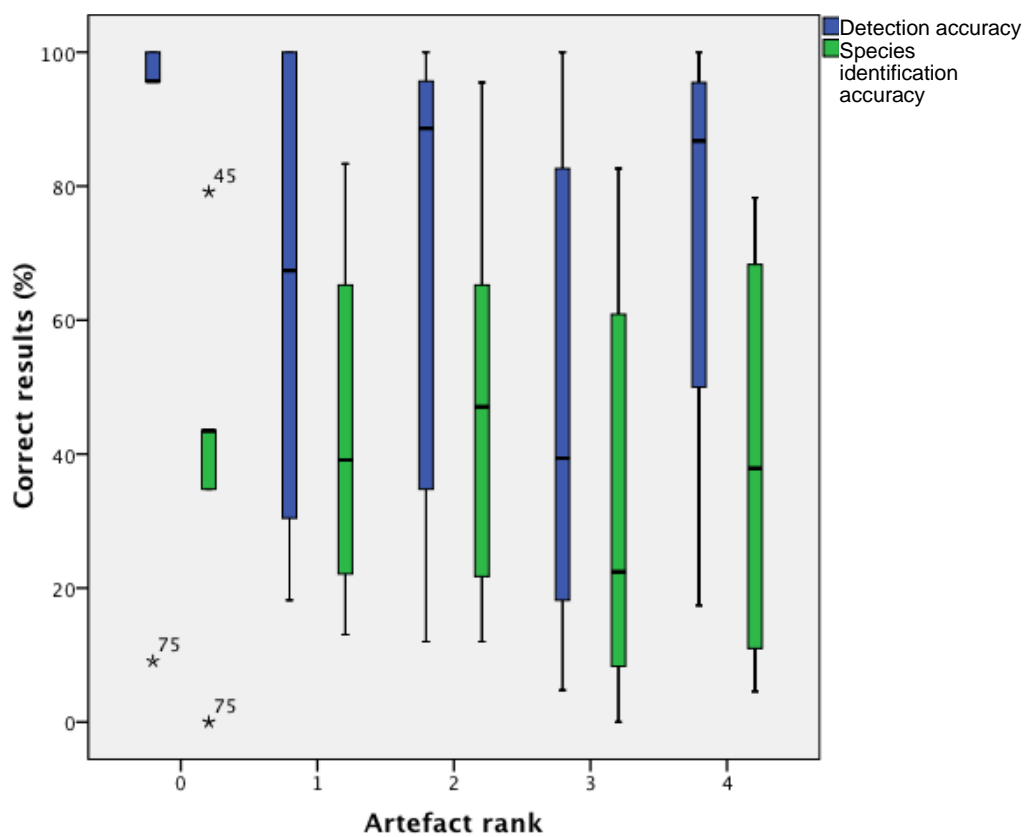


Circles indicate outliers

Presence of artefact

Figure 5.16 demonstrates the results of the detection accuracy and species identification accuracy for the different ranks of artefacts present. There was no significant difference for the presence of artefacts on the slide on the detection accuracy ($p=0.606$) and species identification accuracy ($p=0.814$)

Figure 5.16: International group: The effect of the artefact rank on the detection and species identification accuracy in the final assessment



Stars indicate extreme outliers

Comparison of staff undertaking malarial diagnosis by microscopy in the International group

Table 5.14: Results from international group participants for the final assessment stage cases (n=40)

Location	Individual results	Definitive diagnosis			Detection accuracy (%)	Species identification accuracy (%)
		Positive	Negative	Total		
Kenya	Positive	39	0	39	80.0	61.4
	Negative	11	10	21		
	Total	50	10	60		
Hong Kong	Positive	31	0	31	95.0	75.8
	Negative	2	7	9		
	Total	33	7	40		
Ibadan 1	Positive	64	1	65	70.0	44.5
	Negative	35	20	55		
	Total	99	21	120		
Ibadan 2	Positive	61	3	64	65.8	37.4
	Negative	38	18	56		
	Total	99	21	120		
Lebanon	Positive	63	4	67	78.8	68.2
	Negative	16	14	30		
	Total	79	18	97		
Lagos 1	Positive	95	0	95	65.0	25.5
	Negative	70	35	105		
	Total	165	35	200		
Lagos 2	Positive	56	0	56	64.2	29.3
	Negative	43	21	64		
	Total	99	21	120		
Lagos 3	Positive	72	0	72	62.5	33.3
	Negative	60	28	88		
	Total	132	28	160		
Mean					72.7	46.9

Table 5.14 shows that the mean detection accuracy for all individuals was 72.7% (± 8.8), with a species identification accuracy of 46.9% (± 19.6).

Twenty-one participants completed all 40 cases, the results of those that completed less than ten cases have been excluded.

Parasite detection

Only four participants achieved a detection accuracy of higher than 80% in the post assessment stage, the highest of which was 95%. The lowest detection accuracy was 57.5%.

Parasite speciation

The species identification accuracy was lower than the detection accuracy; with the highest species identification accuracy achieved 75.8% for the 33 positive cases. Sixteen participants had a species identification accuracy of less than 50%, the lowest of which being 18.2%.

Experience of laboratory staff

The results for experience were not significant for the detection accuracy ($p=0.142$) or the species identification accuracy ($p=0.141$)

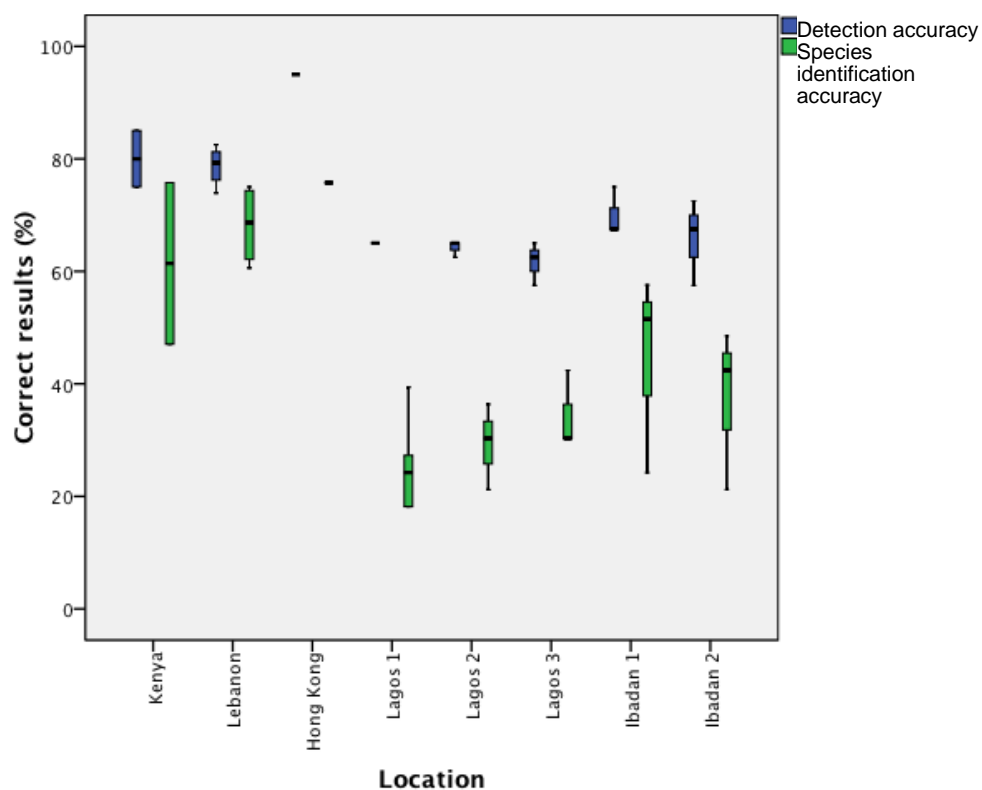
Training of laboratory staff

There was no significant difference of the time since last training occurred on the detection accuracy ($p=0.088$) or the species identification accuracy ($p=0.060$).

Location

The locations of the participants' laboratories were taken into account to see if they had an influence on the diagnosis made. The results of the individuals involved and their locations are shown in figure 5.17.

Figure 5.17: International group: The relationship between the location and the results for detection and species identification accuracy in the final assessment



The results of those from laboratories involved in EQA schemes were better with higher accuracies and species identification accuracies. There was a significant difference in the detection accuracy ($p=0.009$) and the species identification accuracy ($p=0.025$) for the location of the participants.

5.6.2 UK group final assessment results

Of the 34 participants that began the initial assessment stage, there were 25 participants that started the final assessment stage. Sixteen participants have completed all 40 cases in the final assessment stage.

Table 5.15: The detection of parasites in the final assessment stage cases (n=40) for the UK participants group

Definitive diagnosis	n (=40)	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)
Negative	7	94.8	90.9	N/A	N/A
<i>P. falciparum</i>	24	97.3	30.0	80.6	60.0
<i>P. vivax</i>	3	81.5	44.4	37.7	52.1
<i>P. ovale</i>	3	96.8	5.3	55.4	42.1
<i>P. malariae</i>	2	92.1	15.8	71.5	27.1
Mixed infection	1	100.0	N/A	29.4	N/A

N/A Not applicable

Table 5.15 shows the detection accuracy of malaria diagnosis over the 40 cases was 95.5% (± 8.7). Determining the correct species present proved to be a more difficult task, the species identification accuracy was 72.4% (± 24.3).

Table 5.16 demonstrates performance on the individual cases in the final assessment by the UK group.

Table 5.16: The performance on the 40 individual cases in the final assessment by the UK group

Case	Case result	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
41	Negative	92.0			-	3	2
42	<i>P. falciparum</i>	95.8	91.7		3	1	1
43	<i>P. falciparum</i>	100.0	91.7		1	4	2
44	<i>P. falciparum</i>	95.2	90.5	Yes	2	4	2
45	<i>P. falciparum</i>	100.0	100.0		3	0	1
46	<i>P. ovale</i>	95.7	60.9		1	1	3
47	Negative	90.9		Yes	-	2	2
48	<i>P. falciparum</i>	100.0	95.2		3	1	1
49	<i>P. falciparum</i>	70.0	40.0	Yes	2	4	3
50	<i>P. falciparum</i>	100.0	77.3		2	2	2

Case	Case result	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
51	<i>P. falciparum</i>	100.0	95.8		3	2	1
52	<i>P. falciparum</i>	100.0	100.0		3	1	1
53	Negative	95.7			-	2	1
54	<i>P. falciparum</i>	100.0	100.0		2	3	1
55	<i>P. falciparum</i>	100.0	91.3		2	0	1
56	Negative	95.7			-	2	1
57	<i>P. falciparum</i>	100.0	87.0		3	1	1
58	<i>P. falciparum</i>	100.0	100.0		2	3	1
59	<i>P. falciparum</i>	100.0	63.6		3	0	1
60	<i>P. falciparum</i>	100.0	63.6		3	0	1
61	<i>P. falciparum</i>	95.0	80.0		1	2	2

Case	Case result	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
62	<i>P. falciparum</i>	95.0	95.0		1	3	2
63	<i>P. malariae</i>	84.2	57.9	Yes	1	3	2
64	Negative	100.0			-	3	2
65	<i>P. falciparum</i>	100.0	68.4		1	3	2
66	<i>P. ovale</i>	100.0	73.7		1	1	2
67	<i>P. falciparum</i>	89.5	42.1		2	1	2
68	Negative	94.7			-	2	2
69	<i>P. vivax</i>	100.0	63.2		2	2	2
70	<i>P. falciparum</i>	100.0	79.0		2	3	2
71	<i>P. vivax</i>	55.6	11.1	Yes	2	3	2
72	<i>P. falciparum</i>	94.7	52.6	Yes	2	3	3

Case	Case result	Detection Accuracy (%)	Species identification accuracy (%)	Thick film	Parasite density	Artefacts	Rank
73	<i>P. falciparum</i>	100.0	95.0		1	2	2
74	<i>P. malariae</i>	100.0	85.0		1	3	2
75	<i>P. ovale</i>	94.7	31.6		1	0	3
76	<i>P. falciparum</i>	100.0	52.6		1	2	3
77	<i>P. falciparum</i> and <i>P. ovale</i>	100.0	29.4	Yes	2	4	3
78	Negative	94.7			-	3	2
79	<i>P. falciparum</i>	100.0	84.2		1	1	3
80	<i>P. vivax</i>	88.9	38.9	Yes	1	3	3
Average		95.5	72.4				

False negatives

There were a number of false negative results, the reasons for these were split into the following categories.

Low parasite density

There were 13 cases at low parasite density, six of which had some false negatives. For cases 46, 61, 62, and 75 one individual missed the presence of parasites.

Thick films

The majority of false negatives were seen on the thick film. There were five out of six cases in total in which parasites were missed on the thick film. False negative results were seen in the following cases 44 (1), 49 (6), 63 (3), 67 (2), 71 (8), 72 (1) and 80 (2) (brackets indicate the number of false negative cases identified).

Case 67 was also found to be negative by two participants. The case was a thin film, with medium parasite density and few artefacts present.

False positives

Of the seven negative cases there were six in which parasites were falsely identified. Cases 53, 56, 68 and 78 were identified as having parasites present by one individual and case 41 by two. Case 47 a thick film was also identified as positive by two individuals.

Incorrect species

The lowest species identification accuracy was seen in case 71 a *P. vivax* thick film, incorrectly identified by eight participants. The other *P. vivax* case, case 69 was also identified as a different species by seven participants, five as *P. ovale* and two others as a mixed infection that included *P. vivax*.

Artefacts appeared to have had little influence on the diagnosis made, but did have some influence on the species determination.

Low parasite density

Of the 12 low parasite density cases used, 11 cases had problems with speciation. Case 80 a *P. vivax* thick film had eight individuals that incorrectly determined the species. Case 63 was a *P. malariae* thick film that was diagnosed by two individuals as *P. ovale*, two as *P. falciparum* and one mixed infection. Case 74 the *P. malariae* thin film was incorrectly identified by three participants.

All three *P. ovale* cases were present at low parasite densities. Case 46 was identified as *P. vivax* by eight participants, case 66 as *P. vivax* by five participants and case 75 as *P. vivax* by six participants and *P. falciparum* by one participant.

There were six *P. falciparum* cases at low parasite density which had the incorrect species determined, cases 43 (1), 61 (2), 65 (6), 73 (1), 76 (8) and 79 (2) (brackets indicate the number of incorrect species identified).

Thick films

Two *P. vivax* cases 71 and 80 had the incorrect species determined by eight and nine individuals respectively. Case 63 a *P. malariae* case was misdiagnosed by five participants.

Of the three *P. falciparum* cases, case 44, 49 and 72 had the incorrect species determined by one, six and eight participants respectively.

Comparison of cases used in the final assessment for the UK group

Thick and thin films

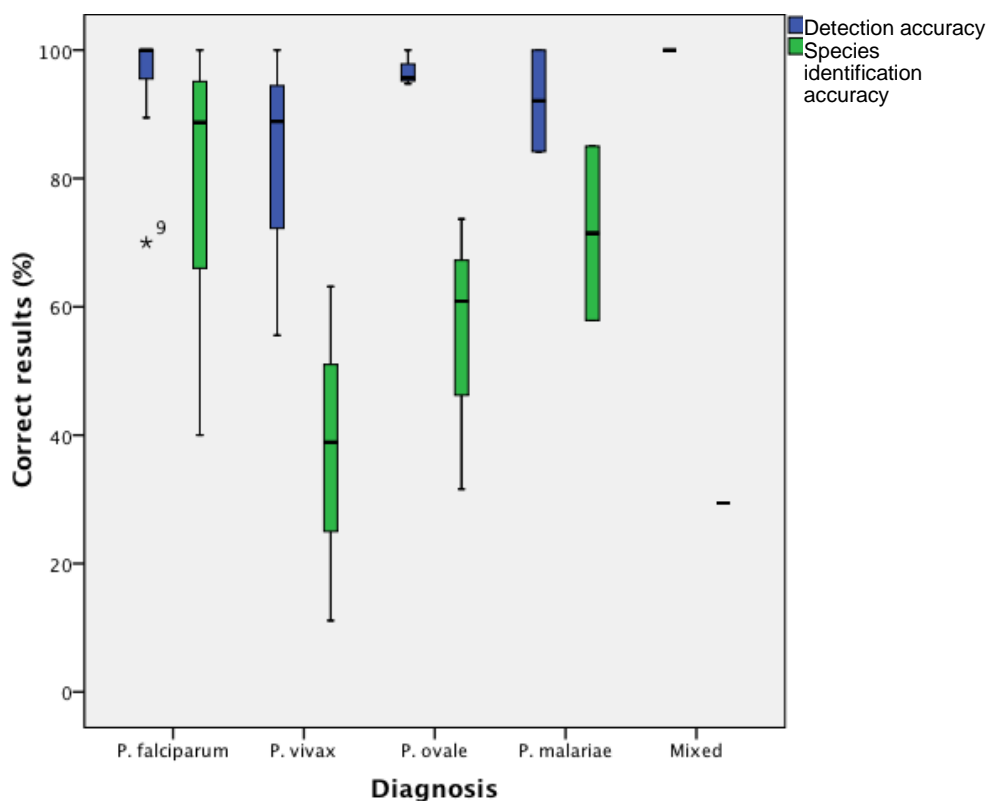
There were eight thick films and 32 thin films in the final assessment. The detection accuracy for thick films was 84.9% (± 15.0) in comparison to thin films 98.1% (± 2.9). The species identification accuracy for the thick films was 45.8% (± 25.0), compared to thin films at 79.7% (± 18.9). There was a highly significant difference for the detection accuracy ($p < 0.001$) and the species identification accuracy ($p = 0.003$) for thick and thin films.

Species

The mixed species case was once again removed from the analysis, due to the small number of cases available. Figure 5.18 shows the comparison between the different species.

Negative samples had a detection accuracy of 94.8% (± 3.2), which was less than for *P. falciparum* and *P. ovale*. *P. falciparum* cases had a detection accuracy of 97.3% (± 6.4), with a species identification accuracy of 80.7% (± 19.0). *P. ovale* also had a high detection accuracy at 96.8% (± 2.8) and a species identification accuracy of 55.4% (± 21.6). This was the second lowest species identification accuracy, higher than that of *P. vivax* at 37.7% (± 26.0).

Figure 5.18: UK group: Comparison of detection and species identification accuracy for the different species present in the final assessment



Stars indicate extreme outliers

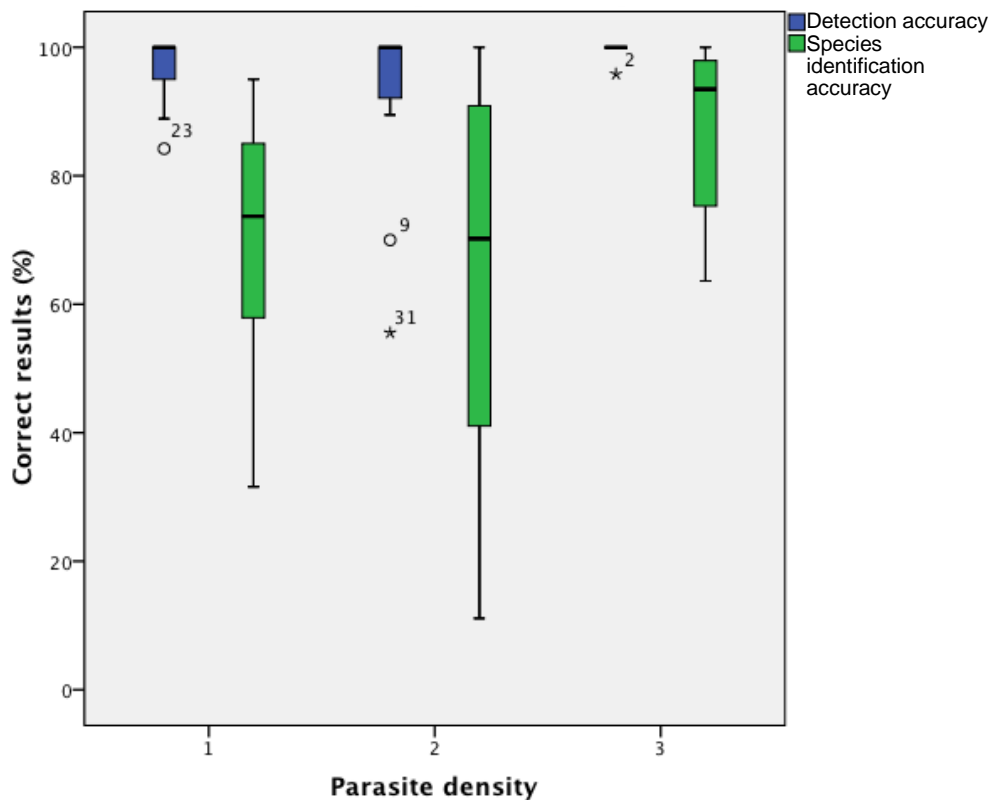
There was a significant difference for the species identification accuracy ($p=0.021$) for the different species. However, there was not a significant

different between the species for the detection accuracy ($p=0.111$) when compared to the species present.

Parasite density of case images

There was not a significant difference between the values for the detection accuracy ($p=0.196$) or the species identification accuracy ($p=0.071$) and the parasite density of the case (figure 5.19). The individuals were equally as good at cases of low parasite density than those of high parasite density.

Figure 5.19: UK group: Comparison of detection and species identification accuracy for the rank of parasite density in the final assessment



Circles indicate outliers, stars indicate extreme outliers

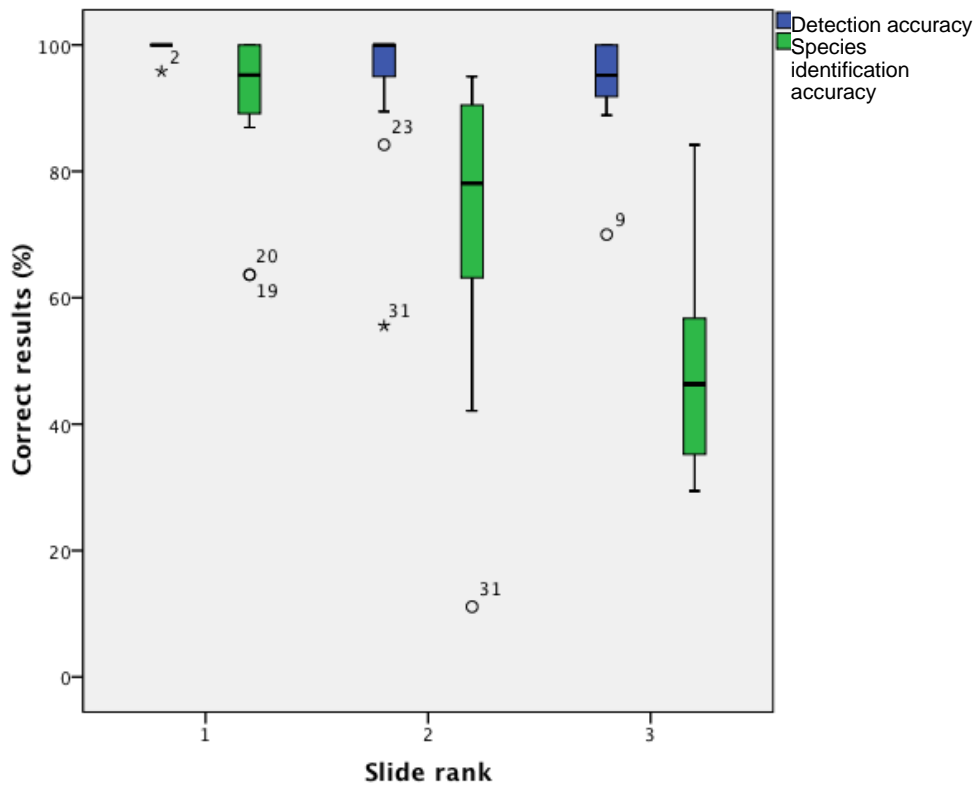
Overall ranking of the microscopic image

Figure 5.20 shows the comparison of the participants' performance on cases of the different ranks. There was a highly significant difference in the species accuracy ($p<0.001$) when compared to the rank of the microscopic image. There was a significant difference for the detection accuracy ($p=0.041$) and the rank of the microscopic image.

Presence of artefact

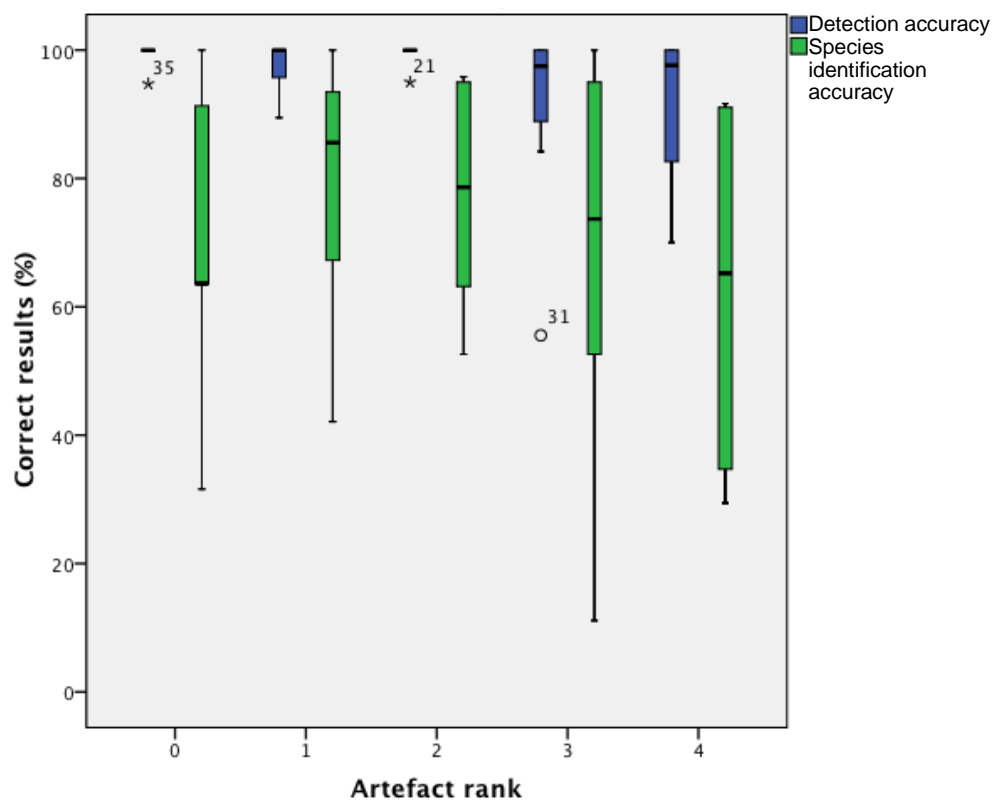
The presence of artefacts appeared to have little difference on the diagnosis made (figure 5.21). There was no significant difference in the detection accuracy ($p=0.555$) or the species identification accuracy ($p=0.879$) when compared to the presence of artefacts.

Figure 5.20: UK group: Comparison of detection and species identification accuracy for the rank of the microscopic image in the final assessment



Circles indicate outliers, stars indicate extreme outliers

Figure 5.21: UK group: Comparison of detection and species identification accuracy when artefacts are present in the final assessment



Circles indicate outliers, stars indicate extreme outliers

Comparison of staff undertaking malarial diagnosis by microscopy in the UK group

Table 5.17: Results from individual participants for the final assessment stage (n=40) for the UK group

Location	Individual results	Definitive diagnosis			Detection accuracy (%)	Species identification accuracy (%)
		Positive	Negative	Total		
1	Positive	159	0	159	97.0	81.8
	Negative	6	35	41		
	Total	165	35	200		
2	Positive	194	4	198	95.7	68.0
	Negative	9	41	49		
	Total	203	45	248		
3	Positive	107	0	107	94.9	76.4
	Negative	5	25	30		
	Total	112	25	137		
5	Positive	30	0	30	92.5	69.7
	Negative	3	7	10		
	Total	33	7	40		
6	Positive	31	1	32	92.5	45.5
	Negative	2	6	8		
	Total	33	7	40		
8	Positive	129	1	130	95.7	78.0
	Negative	5	28	33		
	Total	134	29	163		
Mean					94.7	69.9

Table 5.17 shows the results for the individual participants, the detection accuracy for all individuals was 95.6% (± 3.8) and a species identification accuracy of 73.8% (± 12.8).

Eighteen participants completed all 40 cases, two other participants completed more than 30 cases. The results of those that completed less than ten cases have been excluded.

Parasite detection

The detection accuracy was high, with seven participants achieving 100% detection accuracy. The lowest detection accuracy achieved was 85%, which was obtained after answering 20 cases, incorrectly determining that parasites were absent in three cases. This was the only individual that achieved less than 90% detection accuracy, this individual was in the group with the least experience.

Parasite speciation

The species identification accuracy was as before lower than the detection accuracy, with a detection accuracy of 73.8% (± 12.8). The highest species identification accuracy was by individual UK311 of 96.2%, who completed 32 cases. The lowest species identification accuracy was by UK391 with 48.5%. This participant however correctly determined the presence of parasites in 92.5% of cases.

Location

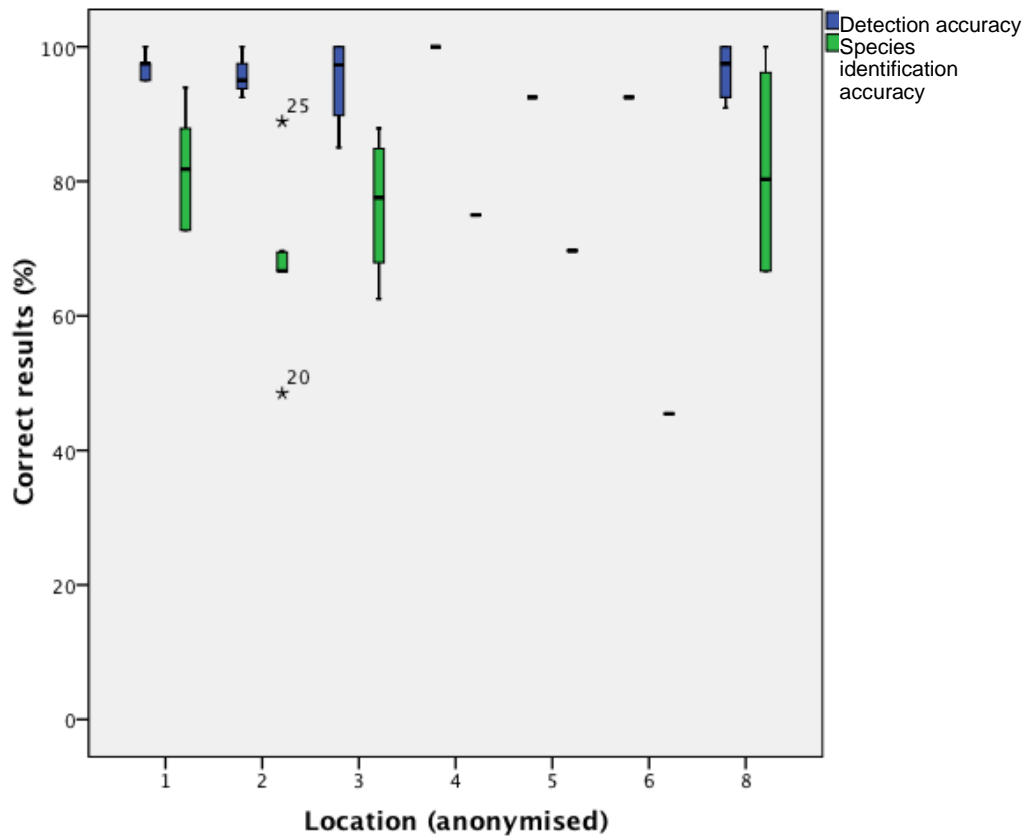
The location results are shown in figure 5.22. There was no significant difference in detection accuracy results ($p=0.618$) between the different hospitals in which the participants were based. The species identification accuracy results were also not significantly different ($p=0.247$) when compared to location.

Experience of laboratory staff

The detection accuracy for less than two years experience (group 1) was 96.6% (± 2.8) and for group two (>5 years experience) was 93.7% (± 4.8). This difference was not significant ($p=0.074$). For species identification

accuracy group one was 71.0% (± 15.5), with group two was 75.2% (± 11.5), this difference was however not significant ($p=0.346$).

Figure 5.22: UK group: The relationship between location and the results of detection and species identification accuracy in the final assessment



Circles indicate outliers, stars indicate extreme outliers

5.6.3. Comparison of UK and International results

The final assessment results for International and UK groups are shown in tables 5.18 and 5.19.

Table 5.18: Results for the 18 participants in the international group for the 40 cases in the final assessment

	Participant responses						
<i>Definitive diagnosis</i>	<i>Total responses</i>	<i>Negative</i>	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>	<i>Mixed</i>
<i>Negative</i>	126	121	2	1	2	0	0
<i>P. falciparum</i>	432	124	198	55	2	16	37
<i>P. vivax</i>	54	35	15	2	1	1	0
<i>P. ovale</i>	54	46	2	0	6	0	0
<i>P. malariae</i>	36	31	4	0	0	0	1
<i>Mixed</i>	18	2	8	1	0	1	6
<i>Total</i>	720	359	229	59	11	18	44

In the final assessment, there were less false positive results for the UK and the international group, compared to those in the initial assessment. There were five false positive results for the international group and six for the UK group. However, there were more false negative results in the final assessment for the international group with 238 (40.1%) instances. For the UK group the number of false negative results fell, with only 14 (3.3%) instances.

Table 5.19: Results for the 13 participants in the UK group for the 40 cases in the final assessment

	Participant responses						
<i>Definitive diagnosis</i>	<i>Total responses</i>	<i>Negative</i>	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>	<i>Mixed</i>
<i>Negative</i>	91	85	1	2	2	1	0
<i>P. falciparum</i>	312	4	255	8	13	22	10
<i>P. vivax</i>	39	8	2	17	5	5	2
<i>P. ovale</i>	39	0	0	14	25	0	0
<i>P. malariae</i>	26	2	0	1	1	21	1
<i>Mixed</i>	13	0	8	0	0	1	4
<i>Total</i>	520	99	266	42	46	50	17

The international participants determined the wrong species in 144 (24.2%) instances, with UK participants having 93 (21.7%) instances. The thick and thin films also once again made differences in the results. Of the wrong species determined by the international group 23 of these were on the thick film. For the UK group 34 instances of the incorrect species were on the thick film.

The thick and thin film also influenced the number of false negative results, 12 of the 14 instances in the UK group were on the thick film. There were also 74 instances of false negative results on the thick film in the international group.

Table 5.20: Detection accuracy and species identification accuracy in the final assessment for both the UK and International group

	International group		UK group	
	Detection accuracy (%)	Species identification accuracy (%)	Detection accuracy (%)	Species identification accuracy (%)
All	66.3	34.7	96.2	74.8
Thick	48.6	18.3	86.5	48.4
Thin	70.7	39.1	98.6	82.0
<i>P. falciparum</i>	71.8	45.8	98.7	81.7
<i>P. vivax</i>	35.2	3.7	79.5	43.6
<i>P. ovale</i>	14.8	11.1	100.0	64.1
<i>P. malariae</i>	13.9	0.0	92.3	80.8
Negative	96.0	N/A	93.6	N/A

N/A= not applicable

Table 5.20 shows that in the final assessment, the detection accuracy was greater for the UK group for every case, the same was also seen for the species identification accuracy. There was a significant difference between the detection accuracy ($p=0.028$) and species identification accuracy ($p=0.001$) in the initial assessment for the UK and International group.

Due to the small number of cases in the individual species groups statistical analysis was not done to compare these for the UK and International groups.

5.7 Comparison of initial and final assessment

5.7.1 International group

There were 18 participants that completed all of the initial and final assessment, allowing their results to be compared and the effectiveness of the training to be assessed.

Table 5.21 shows the results of these individuals for the 80 cases in the entire project. There were differences between the cases and individuals' performance during the initial and final assessment were reviewed.

Table 5.21: Cases from the initial and final assessment and the participant's results for these cases for the International group

Definitive diagnosis	Initial assessment				Final assessment			
	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)
Negative	91.3	16.8	NA	NA	96.0	16.7	NA	NA
<i>P. falciparum</i>	69.4	94.4	42.4	88.9	71.3	94.4	45.8	88.9
<i>P. vivax</i>	85.2	33.3	22.2	33.3	35.2	94.4	3.7	11.1
<i>P. ovale</i>	26.4	72.2	2.8	5.6	14.8	22.2	11.1	22.2
<i>P. malariae</i>	5.6	NA	5.6	NA	13.9	16.7	0.0	0.0
Mixed infection	83.3	NA	0.0	0.0	88.9	NA	0.0	0.0

The eighteen individuals that completed the initial and final assessment achieved a detection accuracy of 68.8% (± 38.7) in the initial assessment and 66.3% (± 36.2) in the final assessment. There was no significant difference in the detection accuracy in the initial and final assessment ($p=0.692$). The species identification accuracy in the initial assessment was 33.3% (± 31.5) and 34.7% (± 29.6) in the final assessment. There was no significant difference in the species identification accuracy ($p=0.879$) in the initial and final assessment.

During the initial and final assessment participants struggled with some particular cases. Described below are the cases in which less than 50% detection accuracy was achieved.

False negative results

Low parasite density

There were 15 out of 25 low parasite density cases in which less than half of the participants made the correct diagnosis.

The main difficulty was seen with *P. ovale* cases, cases 2 (17), 17 (15), 38 (17), 46 (14), 66 (14) and 75 (18) showed these false negative results (brackets indicate the number of false negative cases identified).

Problems were also seen in *P. malariae* cases, in all three cases used. Case 40 was identified as positive by one participant, 63 by four participants and case 74 by one participant.

Six out of a possible 12 *P. falciparum* cases at low parasite density had a high number of false negative results, case 21 (14), 32 (16), 61 (16), 62 (12), 65 (14) and 67 (13), (brackets indicate the number of false negative cases identified)

Thick and thin films

There were three *P. falciparum* thick films out of a possible five that had high numbers of false negative results. There were the following number of false negative results on each case, case 1 (16), 34 (16), 49 (15), 63

(14), 71 (16), (brackets indicate the number of false negative cases identified)

Presence of artefacts

Two cases with artefacts present caused difficulties in determining whether parasites were present. Both of the cases involved were *P. falciparum* cases. Case 28 was a high parasite density infection had 16 false negative results and case 29 showing early trophozoites and chronic granulocytic leukaemia had 17 false negative results. .

Incorrect species

There were 16 cases in which the species was correctly determined by less than half of the participants, the cases with high numbers of false negative results were not included.

There were four *P. vivax* cases in which there was difficulty determining the species present. Cases 8, 10 and 69 only had the correct species determined by two individuals, Case 22 was correctly identified by eight participants.

Similar difficulties were seen with *P. ovale* cases, only one case was identified as positive. Case 24 was identified as positive by 14 participants, however none of these determined the correct species present.

Species identification was also difficult on the two mixed infection cases used. Cases 39 and 77 were both mixed infections of *P. falciparum* and *P. ovale*, however case 77 was a thick film. No participants determined the correct combination of species in either case.

There were nine *P. falciparum* cases in which difficulties in species determination were seen, all of these were cases with cell inclusions. The correct species was determined by six participants for case 11, five participants for case 13 and a similar trend was seen for the remaining cases, cases 18, 25, 30, 55, 57, 59 and 60,

Comparison of cases used for diagnostic assessment

Of the 80 cases used over the initial and final assessments, there were 48 *P. falciparum* cases, 14 negative cases, seven *P. ovale* cases, six *P. vivax* cases, three *P. malariae* cases and two mixed infections. The performance on the cases in the initial and final assessment were compared to the five main categories into which the cases were ranked.

Thick and thin films

There were 15 thick films and 85 thin films, seven thick films (five positives) in the initial assessment and eight (seven positive) in the final assessment. Four of these in the initial and final assessment were *P. falciparum* cases. In the initial assessment, there was a detection accuracy for the thick films of 61.1% (± 36.0), in the final assessment this was 48.6% (± 40.6). Thin films in the initial assessment had a detection accuracy of 70.4% (± 33.7) compared to 70.7% (± 37.5) in the final assessment. The species identification accuracy for thick films was 30.0% (± 33.9) in the initial assessment and 18.3% (± 30.7) in the final assessment. The species identification accuracy for thin films in the initial assessment was 33.7% (± 31.9) compared to 39.1% (± 28.3) in the final assessment.

Figure 5.23, demonstrates the detection accuracy shown on thick and thin films in the initial and final assessment.

Figure 5.23: International group: Comparison of the detection accuracy on thick and thin films in the initial and final assessments

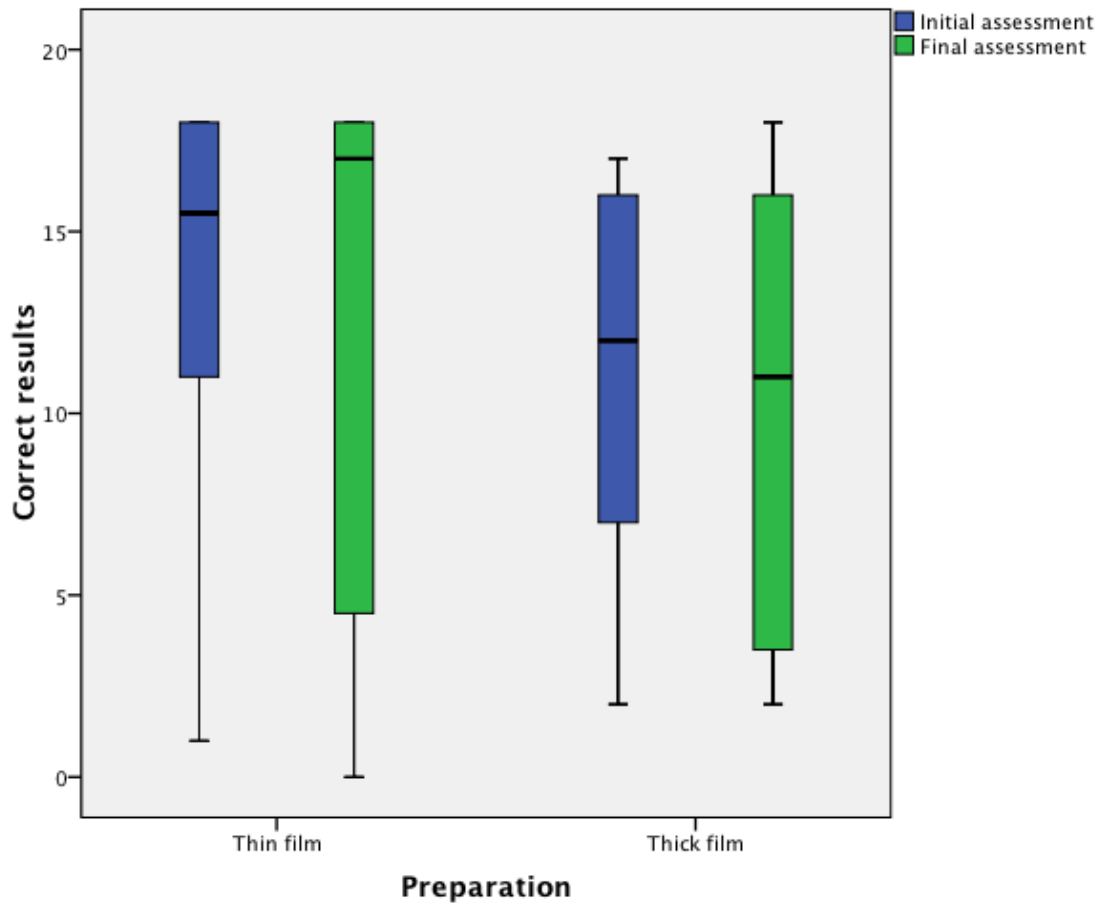
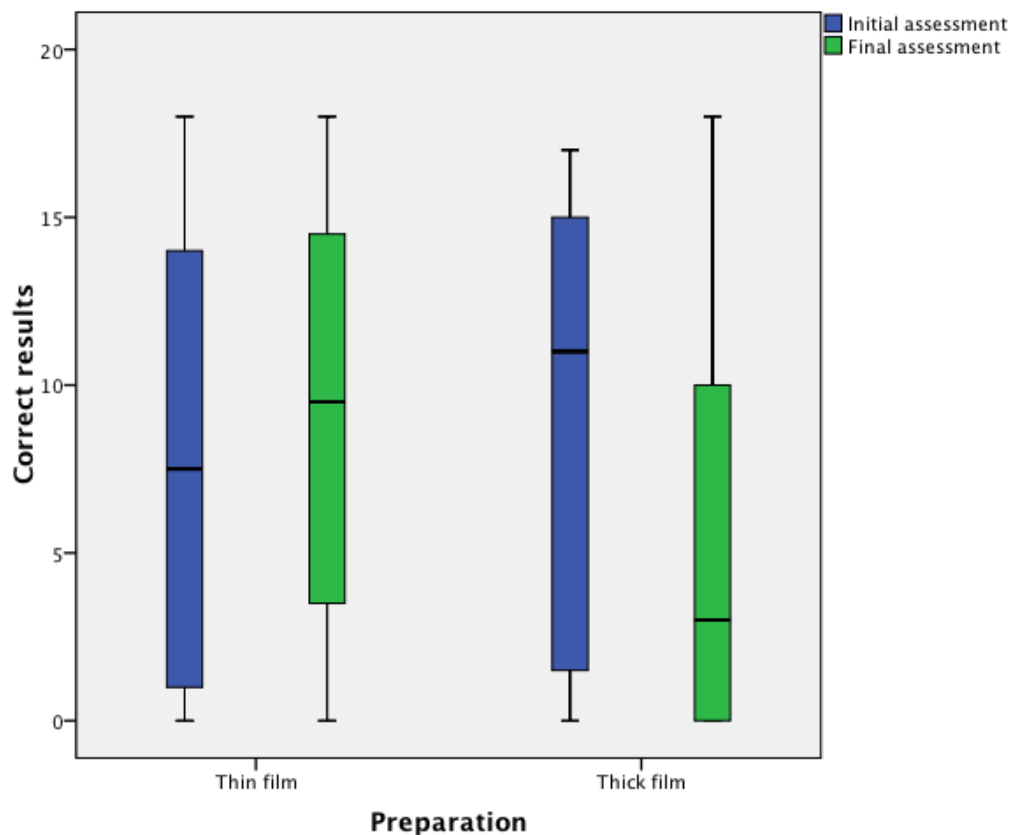


Figure 5.24 compares the species identification accuracy of the thick and thin films in the initial and final assessment.

Figure 5.24 shows that the median number of correct results has increased for thin films from the initial to the final assessment, however for the thick films this has fallen although the highest number of correct results has increased.

Figure 5.24: International group: Comparison of the species identification accuracy on thick and thin films in the initial and final assessment



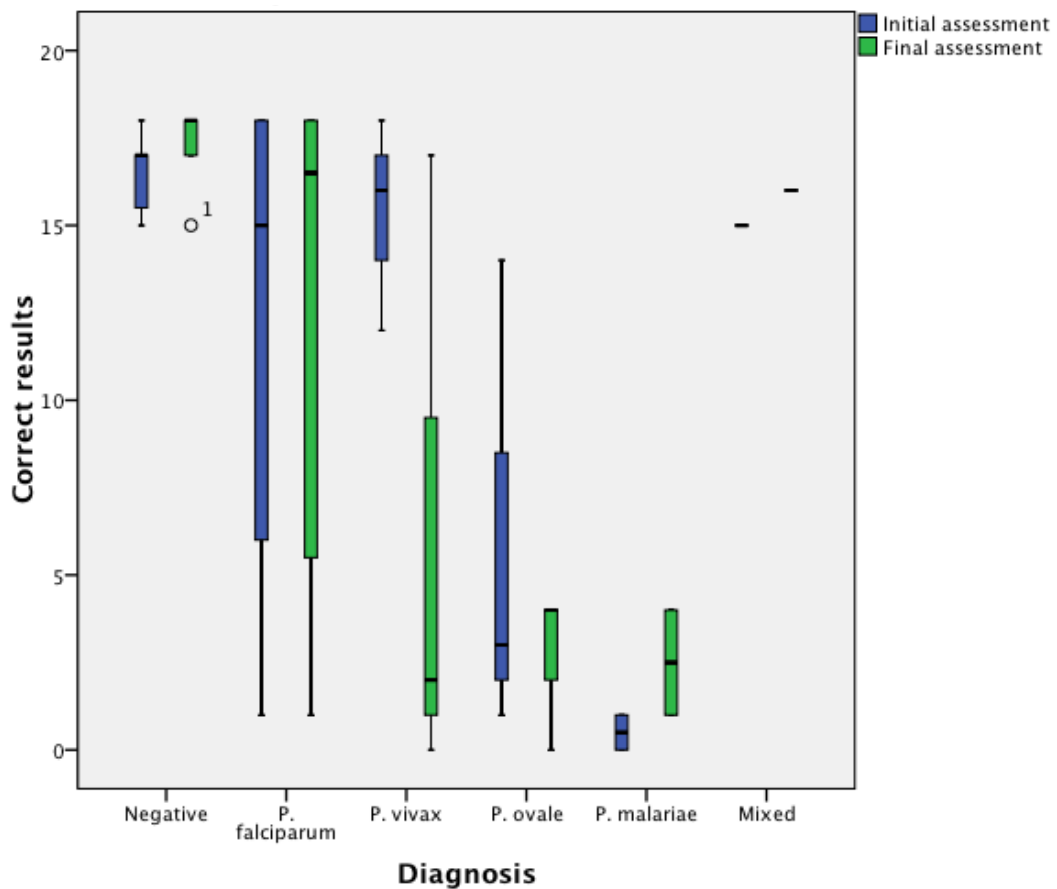
Species

In the initial assessment negative cases had a detection accuracy of 91.3% (± 6.3) compared with the final assessment at 96.0% (± 6.2). *P. falciparum* cases had a detection accuracy of 69.2% (± 36.7) in the initial assessment and 71.8% (± 34.6) in the final assessment. The species identification accuracy for *P. falciparum* cases was 42.1% (± 32.0) in the initial assessment and 45.8% (± 26.9) in the final assessment. In the initial assessment the detection accuracy for *P. ovale* was 26.4% (± 34.7) and the final assessment 14.8% (± 12.8). The species identification accuracy for *P. ovale* in the initial assessment was 2.8% (± 3.2), in the final assessment this was 11.1% (± 11.1). For *P. vivax* the detection accuracy in the initial assessment was 85.2% (± 17.0), in the final assessment this was 35.2% (± 51.6). The species identification accuracy was 22.2% (± 19.2) in the initial assessment and 3.7% (± 6.4) in the final assessment. There was only one *P. malariae* case used in the initial assessment with a detection accuracy of 5.6%, in the final

assessment two cases were used, giving a detection accuracy of 13.9% (± 11.8). The species identification accuracy was 5.6% in the initial assessment and zero in the final assessment.

The comparison of detection accuracy between the different species in the initial and final assessment are shown in figure 5.25. The comparison of the species identification accuracy was shown in figure 5.26.

Figure 5.25: International group: Comparison of the detection accuracy for each case for the different species in the initial and final assessment



Circles indicate outliers

Figure

5.25 shows the median number of correct results has increased between the initial and final assessment for negative cases, *P. falciparum* cases, *P. ovale* cases and *P. malariae*. There was a large drop between the detection accuracy in the initial and final assessment for *P. vivax*.

Figure 5.26: International group: Comparison of the species identification accuracy for each case for the different species in the initial and final assessment

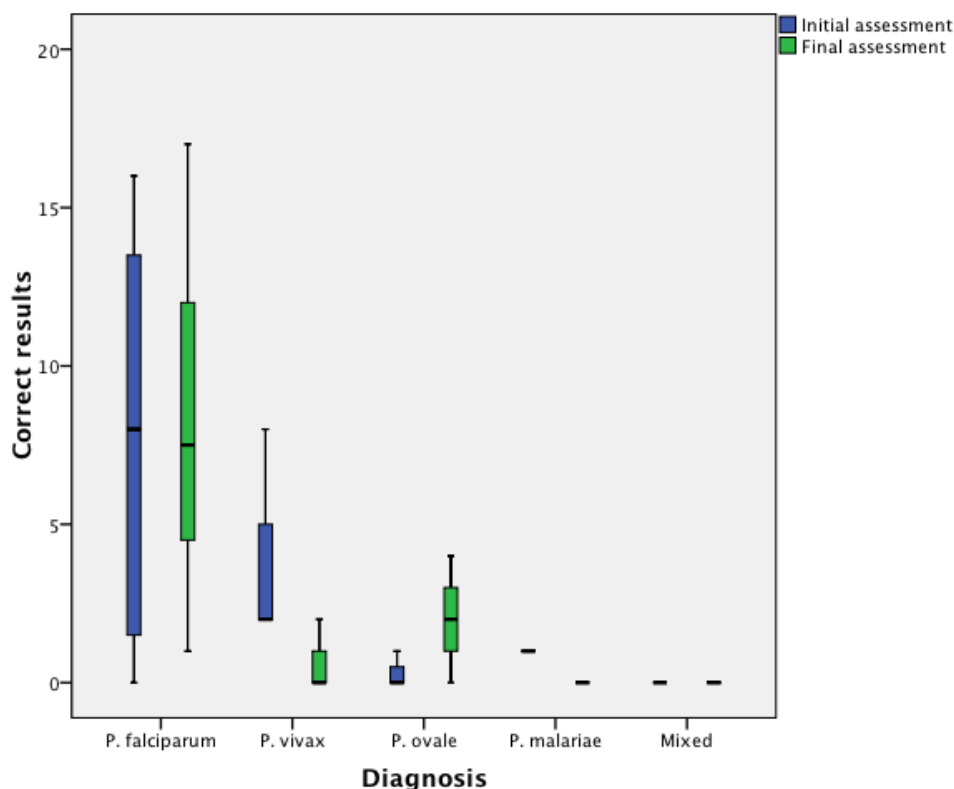


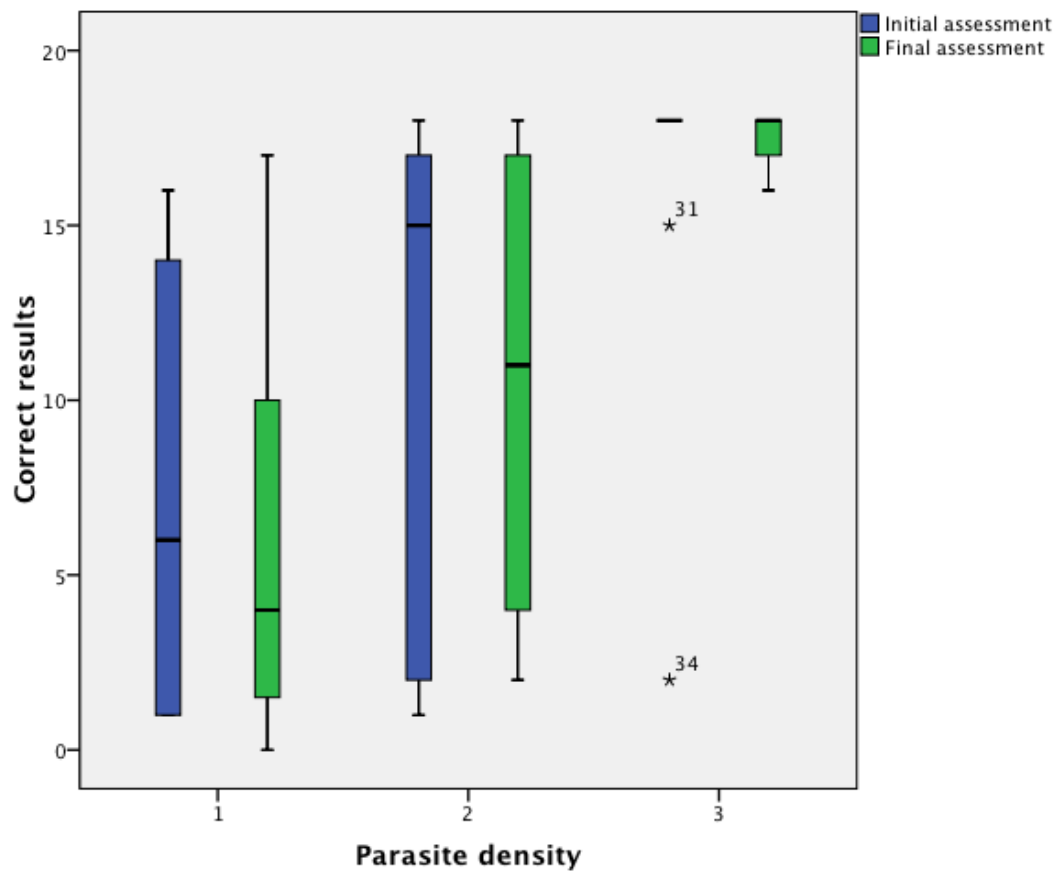
Figure 5.26 shows a decrease in the median species identification accuracy for all species except *P. ovale*, which shows a small increase.

Parasite density

The initial and final assessments demonstrated that the detection accuracy increases as the parasite density increases. In the initial assessment the detection accuracy of diagnosis at the lowest parasite density of less than five cells (rank 1) was 42.1% (± 35.6) and in the final assessment 31.6% (± 34.4). As can be seen from figure 5.19, the number of correct results in the final assessment were lower than in the initial assessment.

For the next parasite density rank 2 (6-49 cells) the detection accuracy in the initial assessment was 64.6% (± 35.6) and in the final assessment 56.2% (± 36.9). Figure 5.27 demonstrates that a similar range of results can be seen, however the median was lower in the final assessment.

Figure 5.27: International group: Comparison of detection accuracy and the parasite density in the initial and final assessment

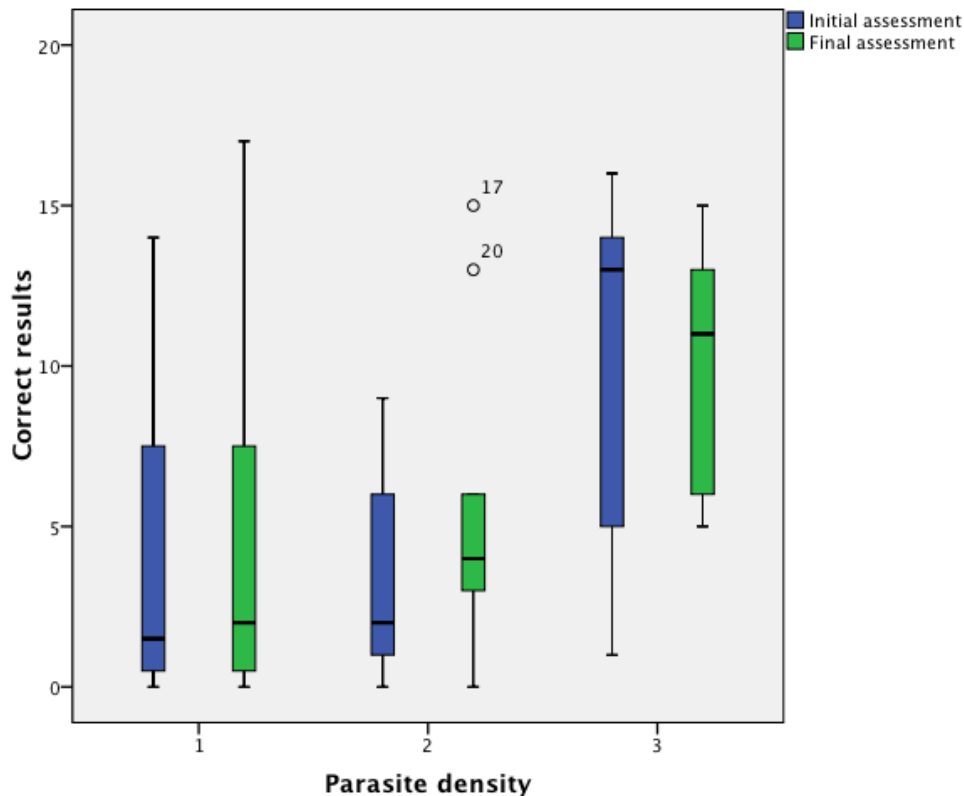


Stars indicate extreme outliers

In the initial assessment for cases with more than 50 parasites present (rank 3) the detection accuracy was 89.4% (± 28.0) and in the final assessment 96.5% (± 5.1) (figure 5.27).

The species identification accuracy for the different parasite density ranks is shown in figure 5.28. There was little difference between parasite ranks one and two in species identification but there was an improvement seen for rank 3.

Figure 5.28: International group: Comparison of species identification accuracy and the parasite density in the initial and final assessment



Circles indicate outliers

The species identification accuracy for rank 1 in the initial assessment was 23.1% (± 29.4) and in the final assessment 22.7% (± 29.4). For parasite density rank 2, the species identification accuracy in the initial assessment was 22.2% (± 23.2) and in the final assessment 31.5% (± 28.1). For parasite density rank 3, the species identification accuracy in the initial assessment was 57.2% (± 31.6) and the final assessment 51.5% (± 25.1).

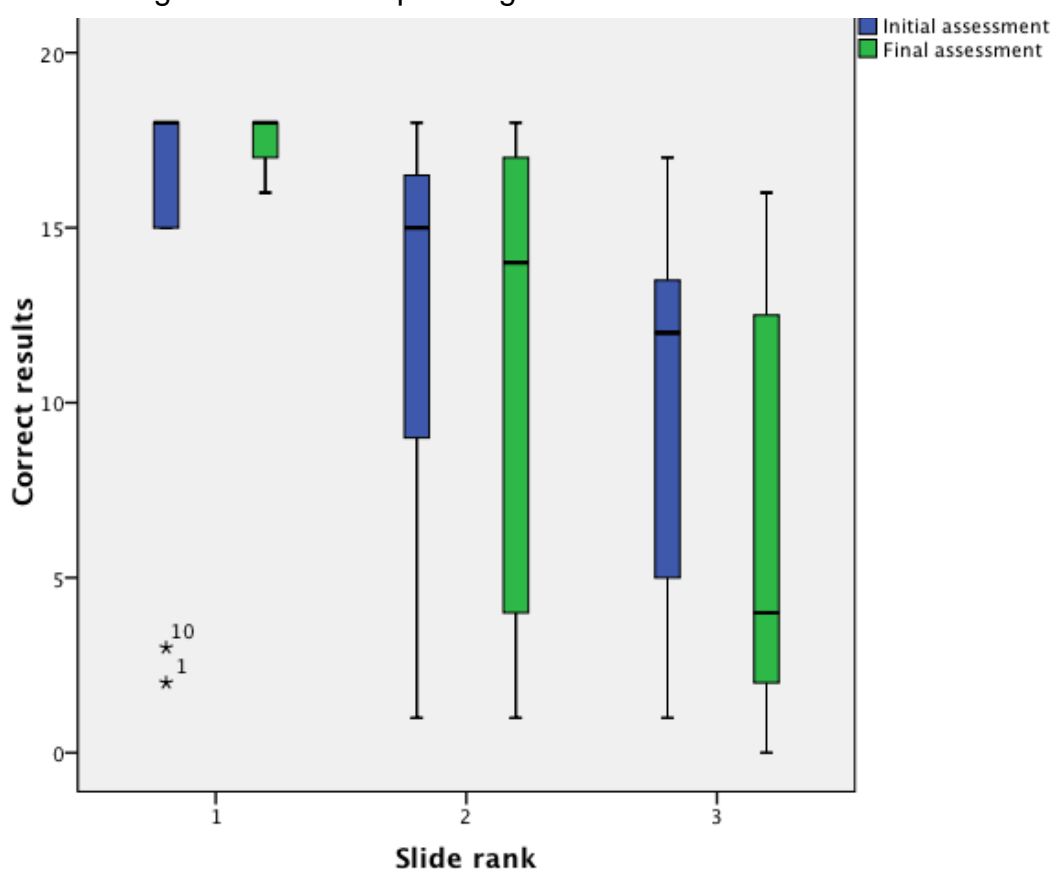
Figure 5.28 shows two results in the final assessment were outside the 95% cut off from the rest of the results. All of these had higher results than the other cases, these cases could be perceived as easier to diagnose, possibly with later stages present making species identification easier.

Overall ranking of the microscopic image

Figure 5.29 demonstrates the trend in the results, when the detection accuracy of the case decreases as the rank of the microscopic image

increases and was deemed more difficult. For rank 1 (easiest), the detection accuracy in the initial assessment was 83.3% (± 31.4) and in the final assessment 97.4% (± 3.7). For rank 2, the detection accuracy in the initial assessment was 64.7% (± 38.0) and in the final assessment 58.5% (± 38.6). For rank 3, the detection accuracy in the initial assessment was 53.2% (± 34.4) and in the final assessment 34.0% (± 36.3).

Figure 5.29: International group: Comparison of the detection accuracy and the ranking of the microscopic image in the initial and final assessment

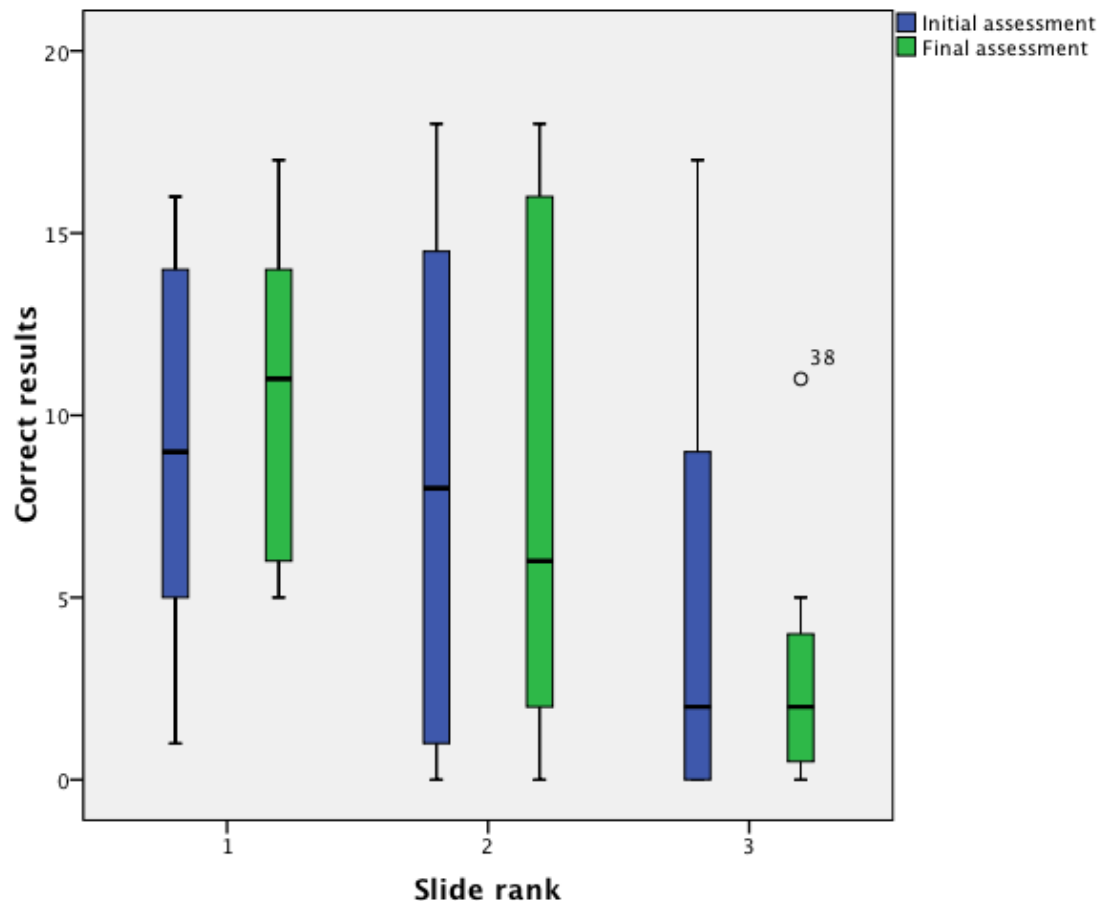


Stars indicate extreme outliers

The species identification accuracy results showed the same trend as the detection accuracy (figure 5.30), with the species identification accuracy decreasing as the rank increased and the cases became more difficult. In species identification accuracy for rank 1 in the initial assessment was 50.5% (± 30.6) and in the final assessment 54.6% (± 19.7). Rank 2 gave a species identification accuracy of 25.2% (± 30.2) in the initial assessment and 30.2% (± 32.2) in the final assessment. Rank 3 gave a species identification

accuracy in the initial assessment of 18.5% (± 25.7) and in the final assessment 15.3% (± 21.0).

Figure 5.30: International group: Comparison of the species identification accuracy and the ranking of the microscopic image in the initial and final assessment



Circles indicate outliers

Figure 5.30 shows the comparison of the species identification accuracy with the rank of the microscopic image in the initial and final assessment.

Presence of artefacts

Table 5.22 shows the detection accuracy and species identification accuracy for the different artefact ranks.

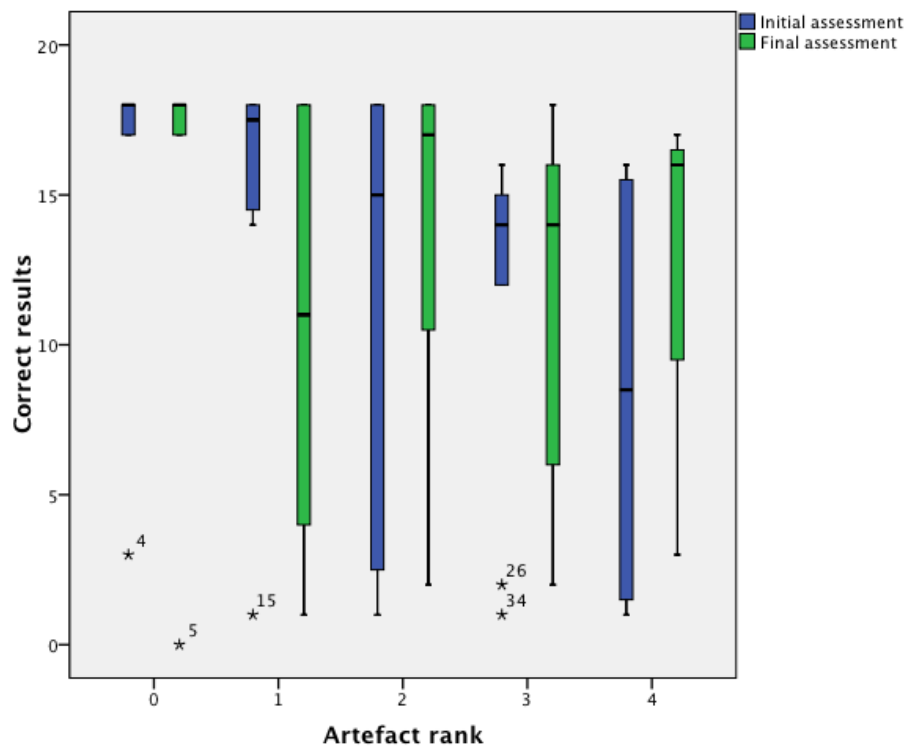
Table 5.22: The detection accuracy and the species identification accuracy of the different artefact rank categories in the initial and final assessment for the International group

	Initial assessment		Final assessment	
	Detection accuracy (%)	Species identification accuracy (%)	Detection accuracy (%)	Species identification accuracy (%)
0	84.9 (± 30.4)	52.8 (± 32.3)	78.9 (± 44.2)	33.3 (± 25.8)
1	82.6 (± 32.3)	42.9 (± 34.7)	59.0 (± 42.8)	36.8 (± 26.6)
2	59.5 (± 46.0)	25.4 (± 26.6)	78.9 (± 33.5)	42.6 (± 34.7)
3	63.0 (± 31.9)	22.2 (± 29.7)	54.3 (± 39.4)	27.8 (± 32.4)
4	86.8 (± 38.7)	24.4 (± 33.9)	72.2 (± 37.1)	37.5 (± 37.0)

The highest variation in results was seen in the initial assessment on cases with an artefact rank of two. This can also be seen on figure 5.31, showing the comparison of the detection accuracy and the artefact rank in the initial and final assessment. Artefact rank zero shows the least variation in results.

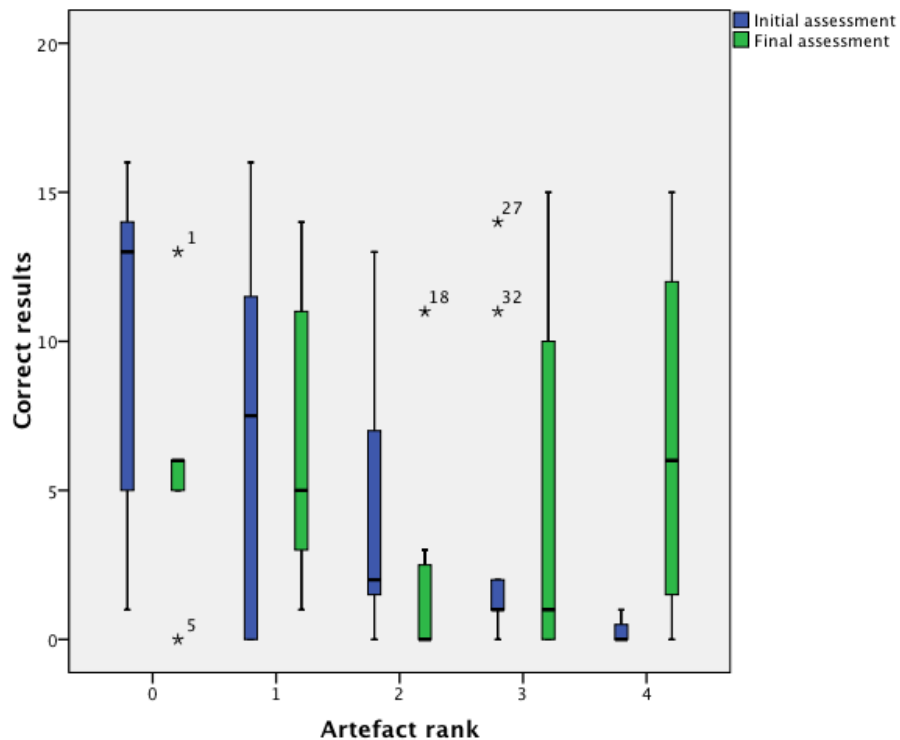
Figure 5.32 shows the comparison of artefacts with the species identification accuracy. There appears to be no influence of the artefact on the species identification accuracy. The median was larger in the initial assessment for rank zero, one and four and two and three in the final assessment.

Figure 5.31: International group: Comparison of the detection accuracy in the presence of artefacts in the initial and final assessment



Stars indicate extreme outliers

Figure 5.32: International group: Comparison of the species identification accuracy in the presence of artefacts in the initial and final assessment



Stars indicate extreme outliers

Comparison of staff undertaking malarial diagnosis by microscopy in the International group

Table 5.23 shows the comparison of the results of members of laboratory staff in the initial and final assessment. Nine participants determined the correct species in more cases in the final assessment, two that had the same number correct and seven that diagnosed fewer cases correctly. Only five participants detected more parasites in the final assessment than the initial.

Individual LT018(D) showed the greatest improvement in results correctly diagnosing 25 cases in the final assessment, compared to 18 in the initial, and the number of incorrect results fell from 16 to seven. LT014 diagnosed 18 cases correctly in the initial assessment, this increased to 23 in the final assessment, and the number of incorrect results fell from 19 to 13. LT005 had eight correct diagnoses in the initial assessment and 14 in the final assessment, incorrect results fell from 19 to 17, showing that for this individual the species identification accuracy increased.

LT001 did not increase the number of correct diagnoses, but had less incorrect results leading to an increased detection accuracy from 19 to 23 cases. LT027 increased the number of correct cases from 23 in the initial assessment to 25 in the final and also decreased the number of incorrect results from 13 to ten.

All other individuals did not improve their diagnoses from the initial to the final assessment.

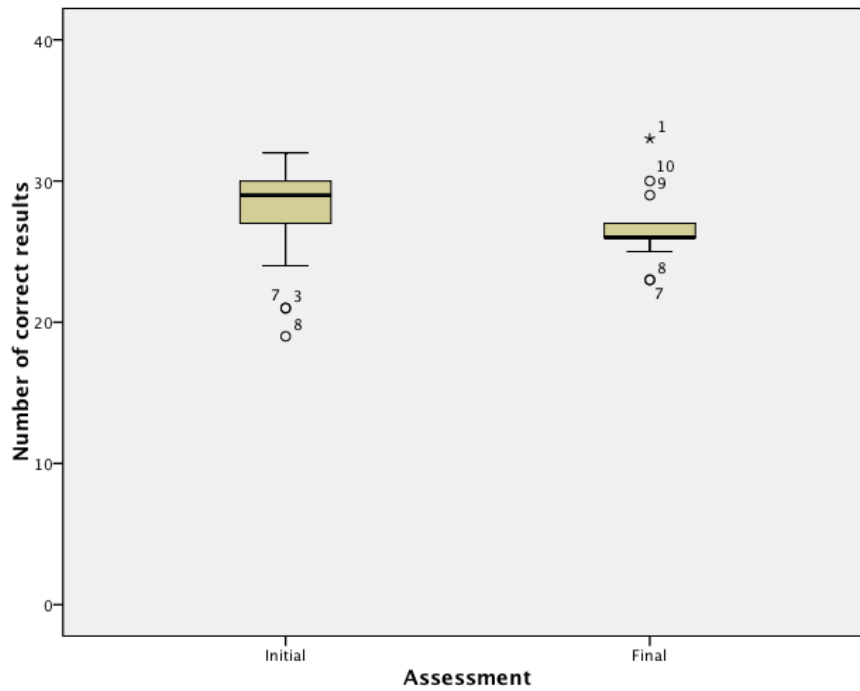
Overall there was no significant difference in the detection accuracy ($p=0.195$) or species identification accuracy ($p=0.451$) between laboratory staff in the initial and final assessment. Figure 5.33 shows the box plot comparing the detection accuracy in the initial and final assessment.

The overall median had fallen between the initial and final assessment. As figure 5.34 demonstrates the correct results achieved by the individuals in the initial and final assessment.

Table 5.23: Comparison of individual participant results in the International group in the initial and final assessment

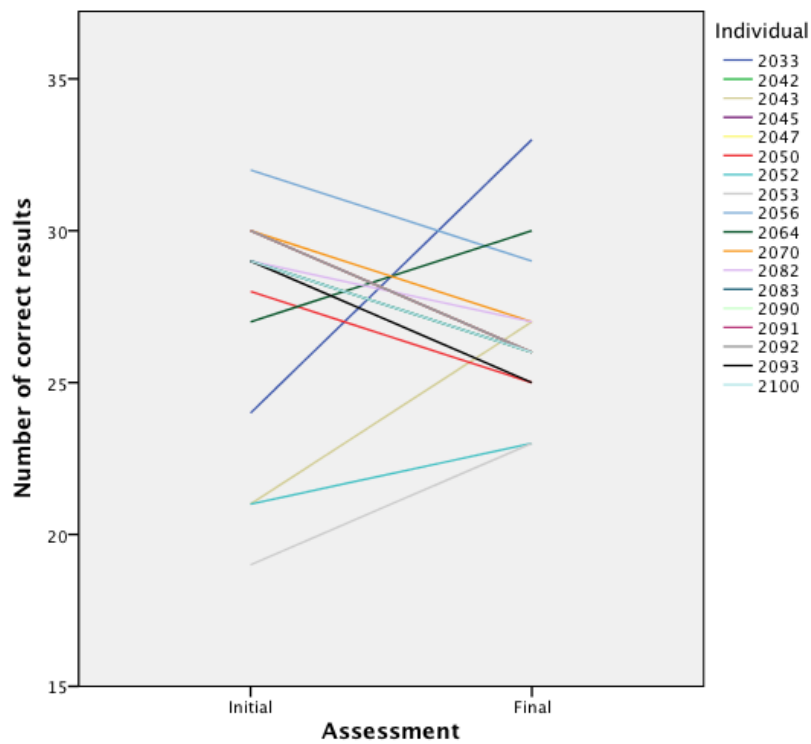
Location	Individual results	Initial assessment					Final assessment				
		Definitive diagnosis			Detection accuracy	Species identification accuracy	Definitive diagnosis			Detection accuracy	Species identification accuracy
		Positive	Negative	Total			Positive	Negative	Total		
Lebanon	Positive	19	2	21	60.0	39.4	28	2	30	82.5	60.6
	Negative	14	5	19			5	5	10		
	Total	33	7	40			33	7	40		
Ibadan 1	Positive	65	0	65	71.7	41.4	64	1	65	70.0	44.5
	Negative	34	21	55			35	20	55		
	Total	99	21	120			99	21	120		
Ibadan 2	Positive	62	9	71	61.7	21.2	61	3	64	65.8	37.4
	Negative	37	12	49			38	18	56		
	Total	99	21	120			99	21	120		
Lagos 1	Positive	92	0	92	75.0	37.1	76	0	76	65.0	27.3
	Negative	40	28	68			56	28	84		
	Total	132	28	160			132	28	160		
Lagos 2	Positive	67	0	67	73.3	33.3	56	0	56	64.2	29.3
	Negative	32	21	53			43	21	64		
	Total	99	21	120			99	21	120		
Lagos 3	Positive	77	0	77	65.6	30.3	72	0	72	62.5	33.3
	Negative	55	28	83			60	28	88		
	Total	132	28	160			132	28	160		
Total					67.9	33.8				68.3	38.7

Figure 5.33: International group: Comparison of the detection accuracy in the initial and final assessment



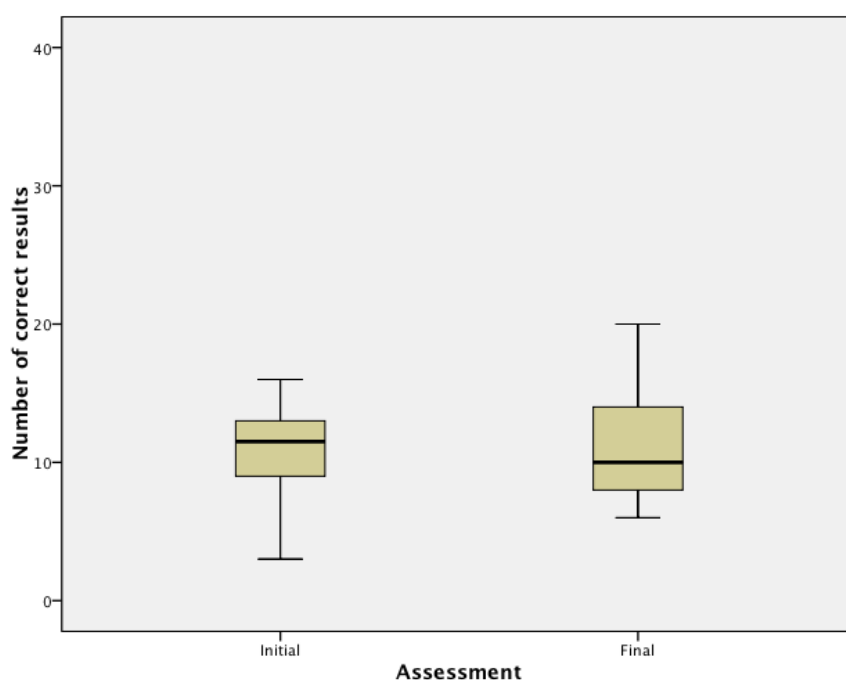
Circles indicate outliers, stars indicate extreme outliers

Figure 5.34: Individual participant correct results in the initial and final assessment in the International group



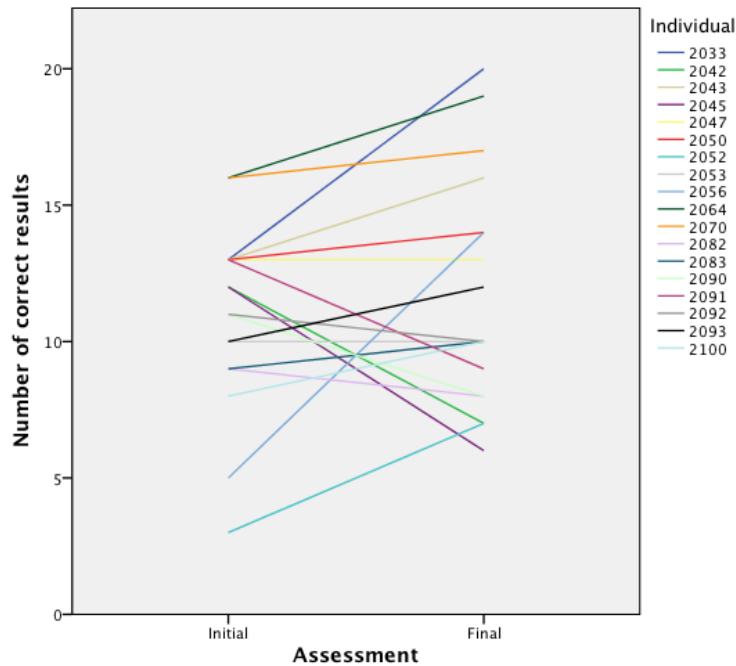
The same trend in the results was evident in the species identification accuracy results, figures 5.35 and 5.36 show the fall in the median and increases and decreases in the number of correct results between the initial and final assessment.

Figure 5.35: International group: Comparison of the species identification accuracy in the initial and final assessment



Some individuals showed an improvement in their results between the initial and final assessment, there were others who were worse. There was no significant difference for either the detection accuracy ($p=0.803$) or species identification accuracy ($p=0.446$) between results of individuals in the initial and final assessment.

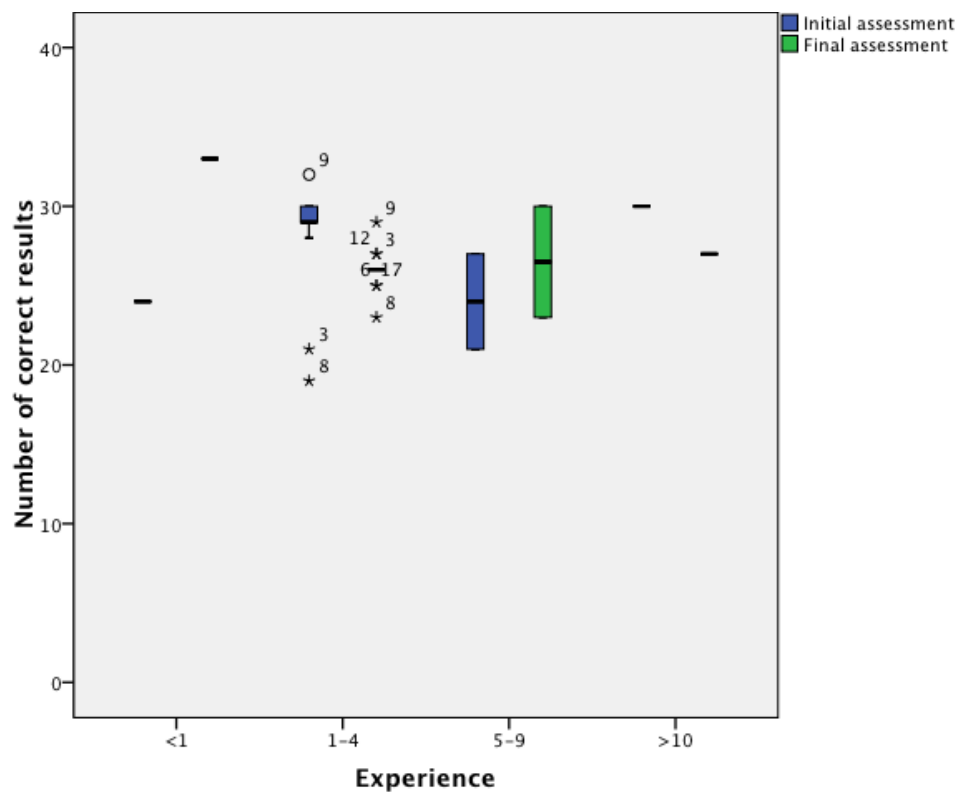
Figure 5.36: International group: Individual correct species results in the initial and final assessment.



Experience of the laboratory staff

The experience of the laboratory staff was expected to influence the effect that the training programme would have on the individual's results. There was only one individual in the less than one and >10 year groups, there were 13 individuals in the 1-4 year group and two in the 5-9 year group. There was variation in the individual results in the initial and final assessment for individuals in the 1-4 year group, in figure 5.37.

Figure 5.37: International group: Comparison of the detection accuracy results and the experience of the individual in the initial and final assessment



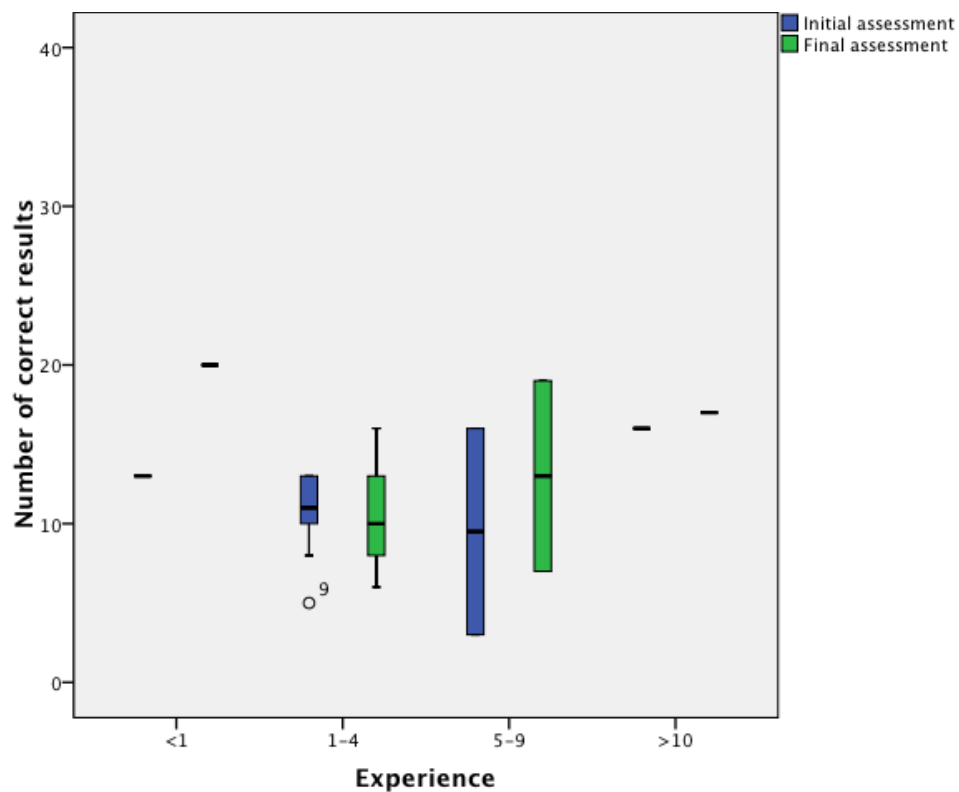
Circles indicate outliers, stars indicate extreme outliers

The individuals with 1-4 years experience showed consistency in the results, with ten individuals agreeing on the results in the initial assessment and six in the final assessment. Figure 5.38, shows the variation in the species identification accuracy between the four experience groups. The species identification accuracy has increased for individuals in the <1, 1-4 and >10 years groups.

The results for species determination show a wider range in the number of correct results achieved, however there are fewer results excluded from the analysis.

There was an increase of one correct case for those with >10 years experience between the initial and final assessment. The individual with less than a years experience improved diagnosis, determining the correct species in 13 cases in the initial assessment and 20 cases in the final assessment.

Figure 5.38: International group: Comparison of the species identification accuracy and the experience of the individual in the initial and final assessment.

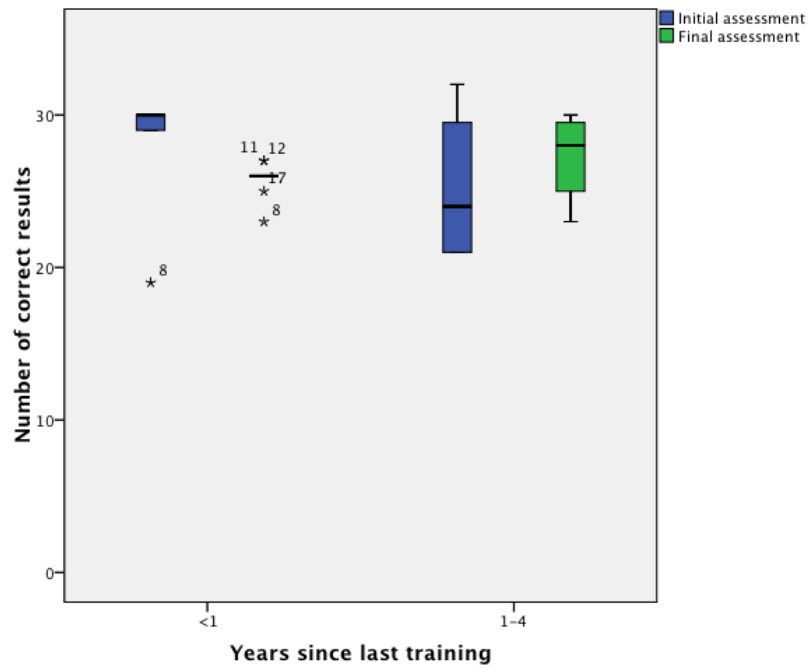


Circle indicates outlier

Training of the laboratory staff

There were 11 individuals in the <1 year group and four in the 1-4 years group. Figure 5.39 shows the results for each group. The species identification accuracy results shown in figure 5.40, show more variation in the number of correct answers when determining the species present, but the trends in the results are the same. Those who have not received training for 1-4 years still had higher species identification accuracy than those who received training less than a year ago.

Figure 5.39: International group: Comparison of the detection accuracy with the training of the individual in the initial and final assessment

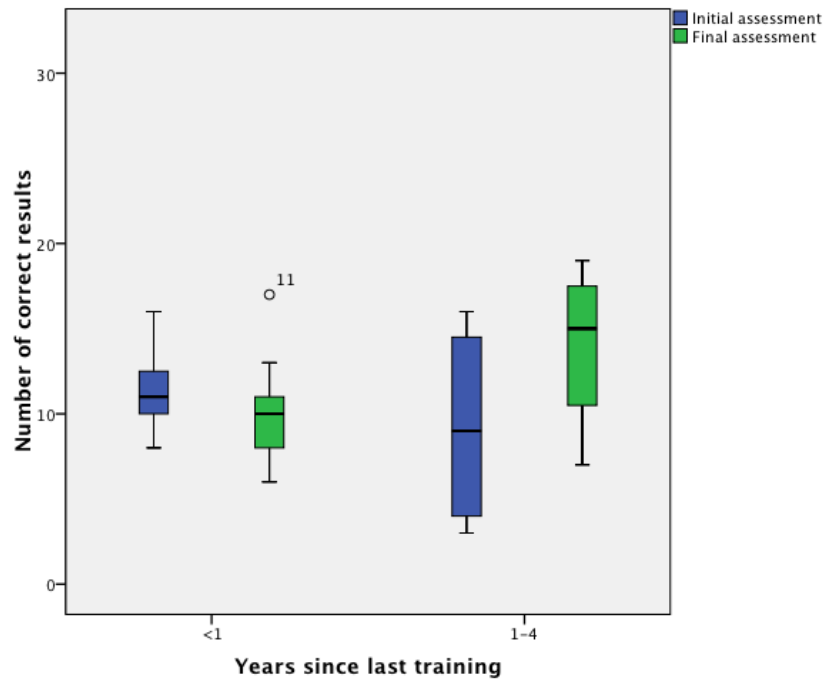


Stars indicate extreme outliers

Species identification

and final assessment

Comparison of the species accuracy with the training of the individual in the initial and final assessment



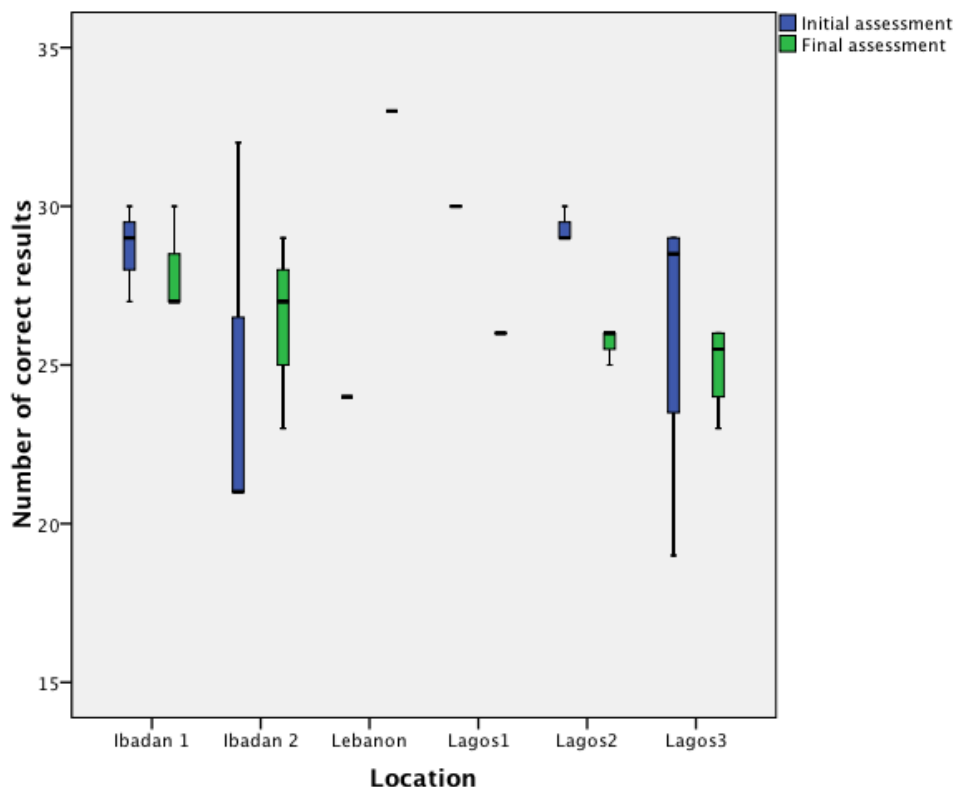
Circle indicates outlier

There was more variation in the 1-4 years group and the median fell for those in the <1 year group.

Location

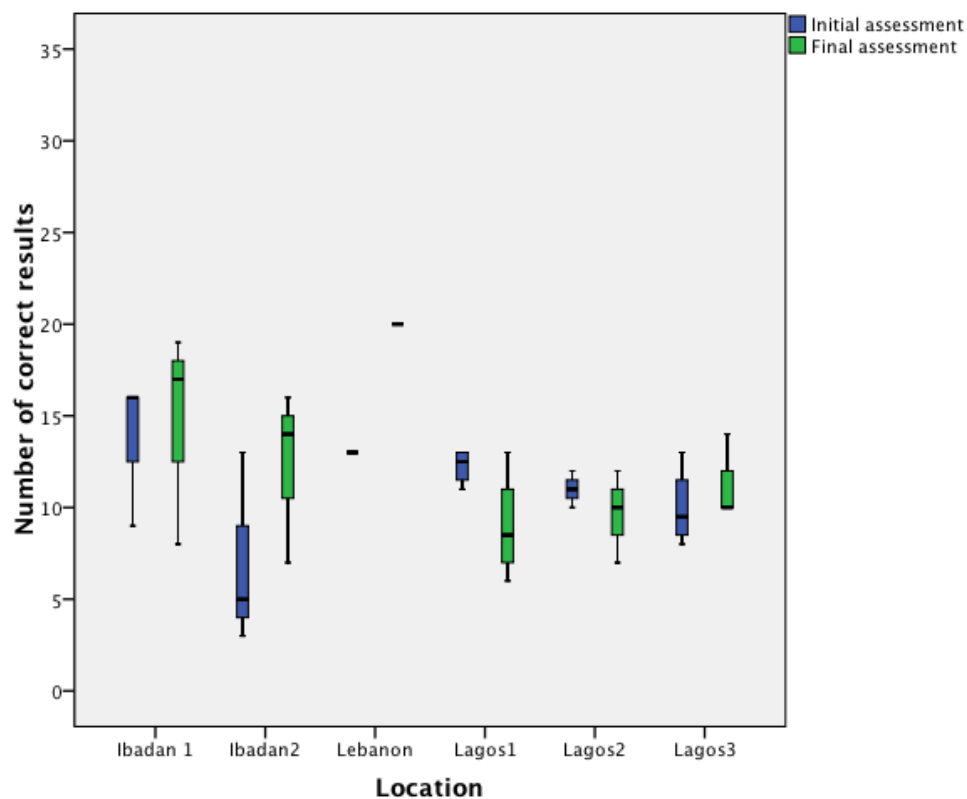
There was only one participant from a laboratory involved in EQA that took part in the entire project and therefore this comparison was excluded. The results could however be compared by the participants locations to see if this had an influence on their results and the use of the training programme. As demonstrated in figure 5.41 there was variation in the results at the different locations.

Figure 5.41: International group: Comparison of the detection accuracy at different participant locations



In the initial assessment the variation at the same location was large especially at Ibadan 2 and Lagos 3. In the final assessment, this variation was smaller. The individual at Lebanon shows the biggest increase in detection accuracy. Improvements in the median between the initial and final assessment were seen at Ibadan 2 and Lebanon, all other locations performed worse in the final assessment. The species identification accuracy was also studied by the location of participants. Figure 5.42 shows the species identification accuracy at the different locations.

Figure 5.42: Comparison of the species identification accuracy at different participant locations in the International group



There was less variation in the results by location for the species identification accuracy than the detection accuracy. There were increases in the median species identification accuracy at Ibadan 1, Ibadan 2, Lebanon and Lagos 3. The highest species identification accuracy was once again seen from the participant at Lebanon.

Comparison of participants results on the same microscopic image

Between the initial and final assessment there were five cases that were repeated, however the microscopic image in the final assessment was an inversion of the one used in the initial assessment. The case comparisons were

1. Case 30 and case 70 (*P. falciparum*)
2. Case 36 and case 72 (*P. falciparum*)
3. Case 4 and case 65 (*P. falciparum*)
4. Case 33 and case 64 (Negative)
5. Case 32 and case 79 (*P. falciparum*)

Table 5.24: Comparison of results from initial assessment cases that were repeated in the final assessment, for the international group

Comparison group	Case	Correct results	Incorrect species	Incorrect results
1	30 (initial)	2	13	3
	70 (final)	13	1	4
2	36 (initial)	11	1	6
	72 (final)	5	6	7
3	4 (initial)	14	2	2
	65 (final)	1	3	14
4	33 (initial)	17	-	1
	64 (final)	18	-	0
5	32 (initial)	1	1	16
	79 (final)	1	-	17

Although the results would be expected to be the same in the initial and final assessment, only comparison image groups one and four showed improvement in the final assessment. For example case 4 has all parasites in the top left corner of the image and was identified as positive by 16 individuals. Speciation however, was better in the final assessment. This improvement could be due to either the positioning of the parasites, with different stages of infection being visible, or an improvement in the participants ability to identify the stages present.

The consistency of the individual's results between the initial and final assessment was analysed, as shown in table 5.25. The agreement between individuals on the two cases was low, with only 52% agreement. There was only agreement by four individuals on the first set of images, cases 30 and 70 and only three individuals agreed with their own results on cases 4 and 65. Only one

individual agreed with all their results on the initial and final assessment. However, 15 of the disagreements led to an improvement in the results.

Table 5.25: Agreement of results between the five repeated cases for the international group

Individual	1	2	3	4	5	Total agree
2033	✓	✗	✗	✓	✗	2
2042	✗	✗	✗	✓	✓	2
2043	✓	✓	✗	✓	✓	4
2045	✗	✓	✗	✓	✓	3
2047	✗	✓	✗	✓	✓	3
2050	✗	✗	✗	✓	✗	1
2052	✓	✓	✗	✓	✓	4
2053	✓	✓	✓	✓	✓	5
2056	✗	✗	✓	✗	✗	1
2064	✗	✓	✗	✓	✓	3
2070	✗	✗	✓	✓	✓	3
2082	✗	✗	✗	✓	✓	2
2083	✗	✓	✗	✓	✓	3
2090	✗	✗	✗	✓	✓	2
2091	✗	✓	✗	✓	✓	3
2092	✗	✗	✗	✓	✓	2
2093	✗	✗	✗	✓	✓	2
2100	✗	✗	✗	✓	✓	2
Total agree	4	8	3	17	15	47/90

Ticks represent agreement. Total number that agree is indicated

5.7.2 UK group

Thirteen participants completed all of the initial and final assessment images, during the time allocated. Other participants completed after this date but had access to the training, so their results were excluded.

Table 5.26 shows the results of these individuals for the 80 cases in the entire project. This allows the differences between the initial and final assessment to be reviewed.

Table 5.26: Comparison of case results in the initial and final assessments (n=80) for the UK group

Definitive diagnosis	Initial assessment				Final assessment			
	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)	Detection accuracy (%)	Detection accuracy range (%)	Species identification accuracy (%)	Species identification accuracy range (%)
Negative	92.3	23.1	N/A	N/A	93.4	15.4	N/A	N/A
<i>P. falciparum</i>	94.0	69.2	78.9	92.3	98.7	15.4	81.7	46.2
<i>P. vivax</i>	71.8	84.6	43.6	53.9	79.5	53.9	43.6	69.2
<i>P. ovale</i>	96.2	15.4	44.2	76.9	100.0	0.0	64.1	53.9
<i>P. malariae</i>	84.6	N/A	30.8	N/A	92.3	15.4	80.8	23.1
Mixed infection	100.0	N/A	53.9	N/A	100.0	N/A	23.1	N/A

N/A= not applicable

The thirteen individuals in the UK group, which completed the initial and final assessment achieved a detection accuracy of 92.3% (± 17.5) in the initial assessment and 96.2% (± 9.4) in the final assessment. There was no significant difference between the detection accuracy results in the initial and final assessment ($p=0.106$). The species identification accuracy in the initial assessment was 69.9% (± 26.3) and in the final assessment 74.8% (± 24.2). There was no significant difference in the species identification accuracy ($p=0.536$) in the initial and final assessment.

There were some cases on which diagnosis was more difficult and individuals had difficulty diagnosing whether parasites were present and what species was present. The cases discussed here had the incorrect diagnosis made by five or more participants.

False negative results

There were only three cases identified as false negative results by five or more participants, all of which were thick films. Case 10 a *P. vivax* thick film was identified as negative by 11 participants. The parasites on this film were located in one corner of the image and were difficult to identify as parasites due to the artefacts present. Case 71 also a *P. vivax* thick film had seven false negative results. Case 34 a *P. falciparum* thick film was identified as negative by nine participants.

Incorrect species

Species determination proved to be more difficult than diagnosing the presence of parasites. There were 15 out of the 84 positive cases which more than five participants identifying the wrong species.

The most difficulty was shown on *P. vivax* and *P. ovale* cases, mainly in determining the difference between these two species, as the morphology is very similar. There were five *P. ovale* cases that showed these problems. Case 2 had 10 participants determining the wrong species, nine of which identified *P. vivax*. Case 17 was identified as *P. vivax* by six individuals. Case 24 was identified as another species by nine participants, six of these being *P. vivax*. Case 66 was

identified by five participants as *P. vivax*. Finally case 75 was identified as *P. vivax* by eight participants.

The same problem was seen for *P. vivax* cases. Case 8 was identified as *P. ovale* by four participants and *P. falciparum* by one. Case 22 was identified as *P. ovale* by three participants and *P. falciparum* by two. Case 71 a thick film was identified as *P. falciparum* by two participants, *P. ovale* by two and *P. malariae* by one. Case 80 also a thick film was identified by *P. malariae* by four participants, *P. ovale* by one and one participants could not identify the species.

P. malariae also caused problems with species identification in the initial assessment on case 40. Six individuals identified this case as *P. vivax*, one as *P. ovale* and one participant did not identify the species present.

Mixed species infections caused a few problems with species identification. However, the species identified was usually correct, although the mixed infection was often missed. Case 39 a *P. falciparum* and *P. ovale* was identified as *P. falciparum* by four participants and *P. falciparum* and *P. vivax* by two participants. Case 77 the thick film showing *P. falciparum* and *P. ovale* mixed infection had a similar pattern. Eight participants identified *P. falciparum* alone, one *P. malariae*, and one as a *P. falciparum* and *P. malariae* mixed infection. However, as speciation is not usually carried out on the thick film, these differences may not be relevant.

Speciation difficulties

All problems with speciation on *P. falciparum* cases were seen in the final assessment. Thick films caused the most problems, with case 49 being identified as another species by five individuals and six identified case 72 also a thick film as another species.

Cell inclusions caused problems in species identification on cases 59 (5), 60 (5), 67 (6) and 76 (6) (brackets indicate the number of incorrect species identified).

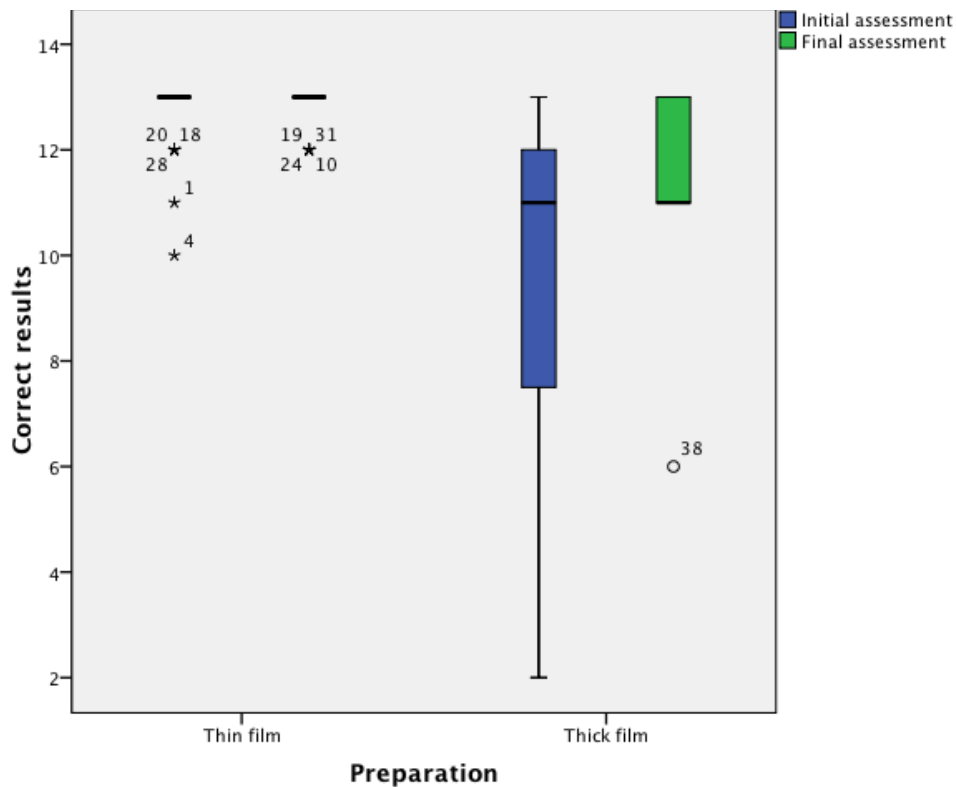
Comparison of cases used in the initial and final assessment

Of the 80 cases used over the initial and final assessments, there were 48 *P. falciparum* cases, 14 negative cases, seven *P. ovale* cases, six *P. vivax* cases, three *P. malariae* cases and two mixed infections. The performance on the cases in the initial and final assessment, were compared to the five main categories into which the cases were ranked.

Thick and thin films

There were 15 thick films and 65 thin films, seven thick films in the initial assessment and eight in the final assessment. In the initial assessment, there were five thick film positive cases, compared to seven in the final assessment. Four of the thick film cases in the initial assessment and four in the final assessment were *P. falciparum* cases. In the initial assessment, there was detection accuracy for the thick films of 71.4% (± 33.7), in the final assessment this was 86.5% (± 17.8). Thin films in the initial assessment had a detection accuracy of 96.7% (± 6.7) compared to 98.6% (± 3.1) in the final assessment. The species identification accuracy for thick films was 52.3% (± 43.0) in the initial assessment and 48.4% (± 28.0) in the final assessment. The species identification accuracy for thin films in the initial assessment was 73.1% (± 21.9) compared to 82.0% (± 17.7) in the final assessment.

Figure 5.43: UK group: Comparison of the detection accuracy on thick and thin films in the initial and final assessment

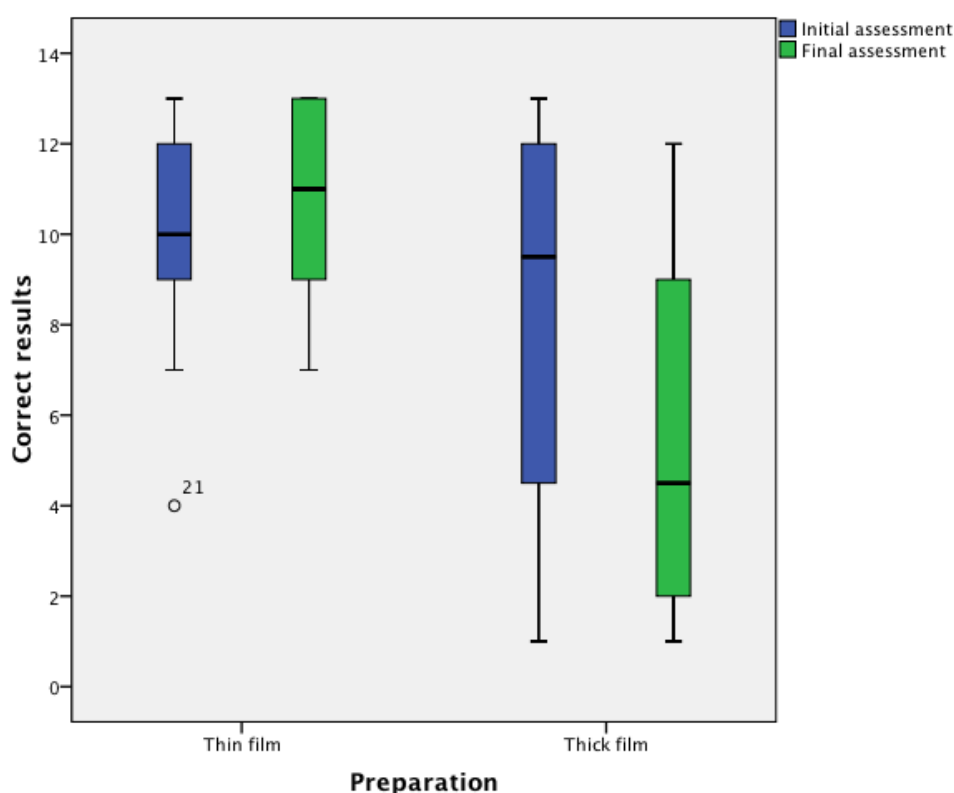


Circles indicate outliers, stars indicate extreme outliers

Figure 5.43, demonstrates the detection accuracy shown on thick and thin films in the initial and final assessment. The detection accuracy on the thin film was higher in both the initial and final assessments. There was no improvement in the detection accuracy or the species identification accuracy between the initial and final assessment.

Figure 5.44 shows that, in the initial and final assessment, there was a slight improvement in the species identification accuracy on the thin film, but a poorer performance on the thick film. The variation in results was also much higher on the thick film than on the thin film, in both the initial and final assessments.

Figure 5.44: UK group: Comparison of the species identification accuracy on the thick and thin films in the initial and final assessment



Circle indicates outlier

Species

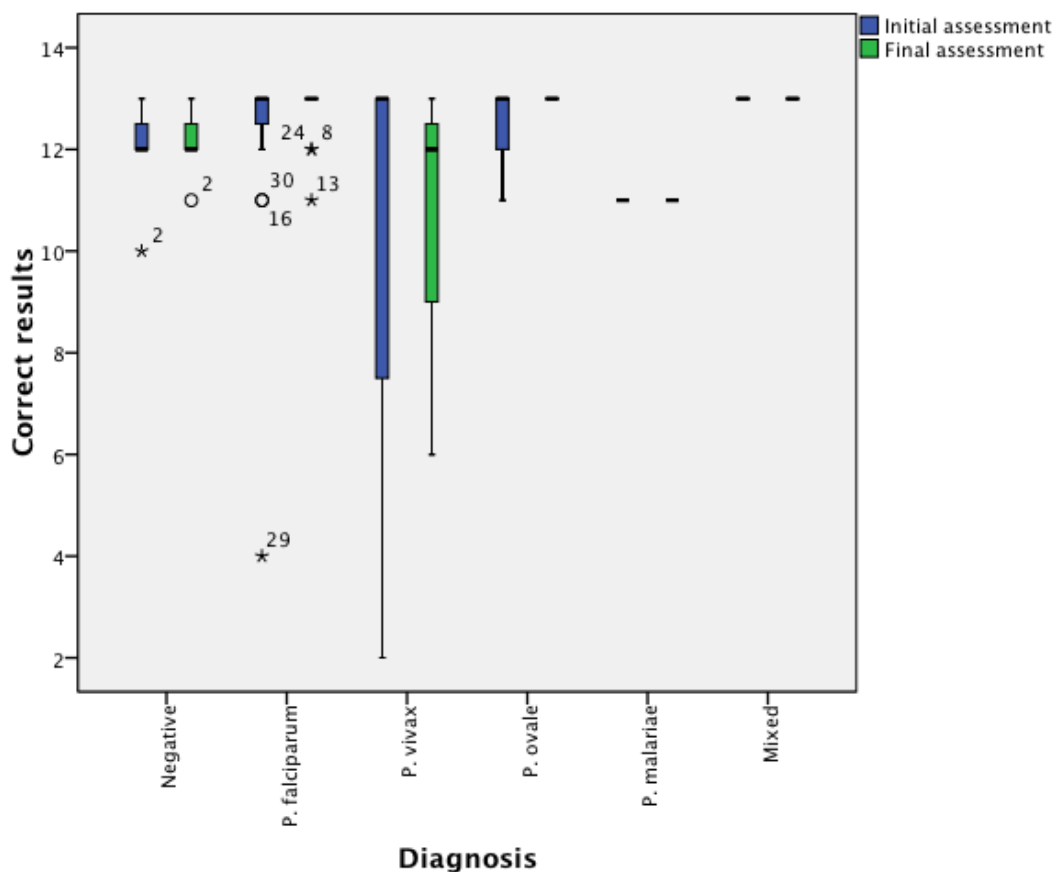
In the initial assessment negative cases had a detection accuracy of 92.3% (± 8.4) compared with the final assessment at 93.6% (± 5.8). *P. falciparum* cases had a detection accuracy of 94.7% (± 15.1) in the initial assessment and 98.7% (± 3.7) in the final assessment. The species identification accuracy for *P. falciparum* cases was 79.6% (± 19.2) in the initial assessment and 81.7% (± 18.0) in the final assessment. In the initial assessment, the detection accuracy for *P. ovale* was 96.2% (± 7.7) and the final assessment 100%. The species identification accuracy for *P. ovale* in the initial assessment was 44.2% (± 32.9), in the final assessment this was 64.1% (± 27.0), if *P. vivax* results were included species identification accuracy could be increased to 98.6%. For *P. vivax* the detection accuracy in the initial assessment was 71.8% (± 48.9), in the final assessment, this was 79.5% (± 29.1). The species identification accuracy was 43.6% (± 31.1) in the initial assessment and 43.6% (± 34.7) in the final assessment. There was only one *P. malariae* case used in the initial assessment

with a detection accuracy of 84.6%, in the final assessment two cases were used, giving a detection accuracy of 92.3% (± 10.9). The species identification accuracy was 30.8% in the initial assessment and 80.8% (± 16.3) in the final assessment.

The comparison of detection accuracy between the different species in the initial and final assessment are shown in figure 5.45. The comparison of the species identification accuracy is shown in figure 5.46.

Figure 5.45 shows very little change in results between the initial and final assessment. There was overall less variation in the results in the final assessment. The detection accuracy of *P. malariae* has improved between the initial and final assessment and there was less variation in the results, showing that individuals agree on the diagnosis in the majority of the cases.

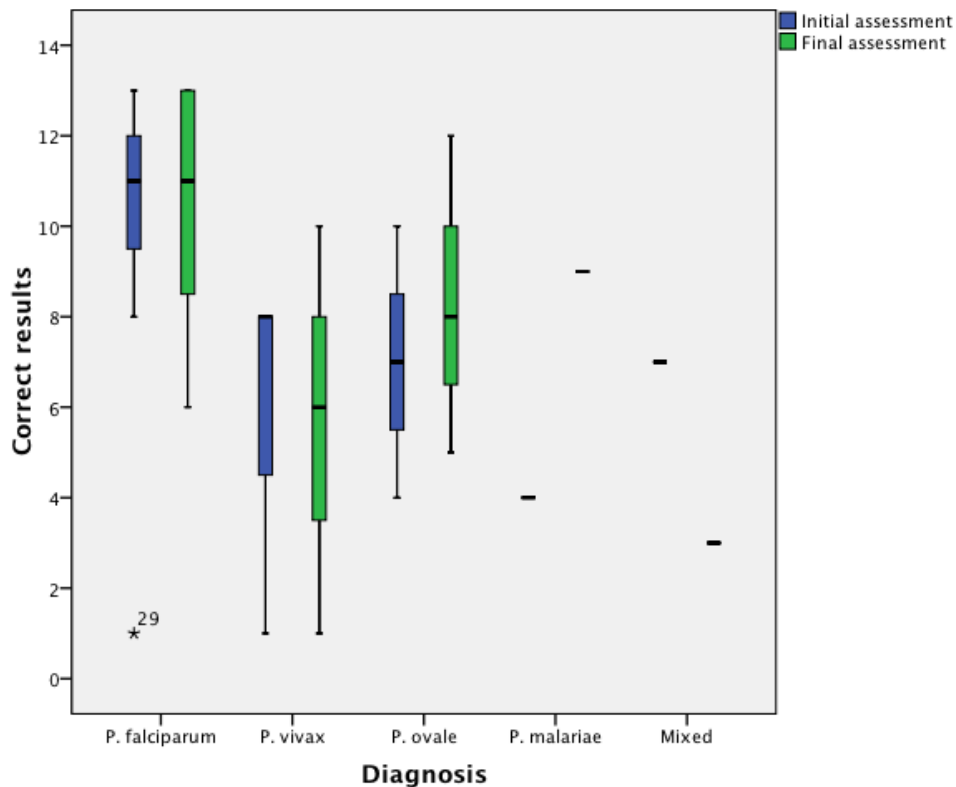
Figure 5.45: UK group: Comparison of the detection accuracy for the different species in the initial and final assessment



Circles indicate outliers, stars indicate extreme outliers

Figure 5.46 shows considerable differences between the species identification accuracy of the different species. The species identification accuracy decreases for *P. vivax* between the initial and final assessment, increases for *P. ovale* and *P. malariae* and was unchanged for *P. falciparum*. The variation seen in results was smaller in the final assessment for *P. ovale* but otherwise the range seen was similar.

Figure 5.46: UK group: Comparison of the species identification accuracy for the different species in the initial and final assessment



Star indicates extreme outlier

The species appears to have an influence on the detection accuracy of diagnosis, however there was little difference to show that the training has influenced this.

Parasite density of case images

The initial and final assessments demonstrated that the detection accuracy increases as the parasite density increases. In the initial assessment the detection accuracy of diagnosis at the lowest parasite density of less than five cells (rank 1) was 87.8% (± 24.2) and in the final assessment 97.6% (± 4.6).

For the next parasite density rank 2 (6-49 cells) the detection accuracy in the initial assessment was 91.6% (± 20.8) and in the final assessment 92.3% (± 18.0).

In the initial assessment for cases with more than 50 parasites present (rank 3) the detection accuracy was 98.5% (± 4.9) and in the final assessment 99.3% (± 2.3).

Figure 5.47 indicates that the parasite density has no effect on the detection accuracy in the initial and final assessment. There was also no difference in the median between the initial and final assessment, however there was a reduction in the variation seen. Any results that are outside of the median are deemed to be outside the distribution due to the small variation in results.

Figure 5.47: UK group: Comparison of detection accuracy and the parasite density in the initial and final assessment

Correct results

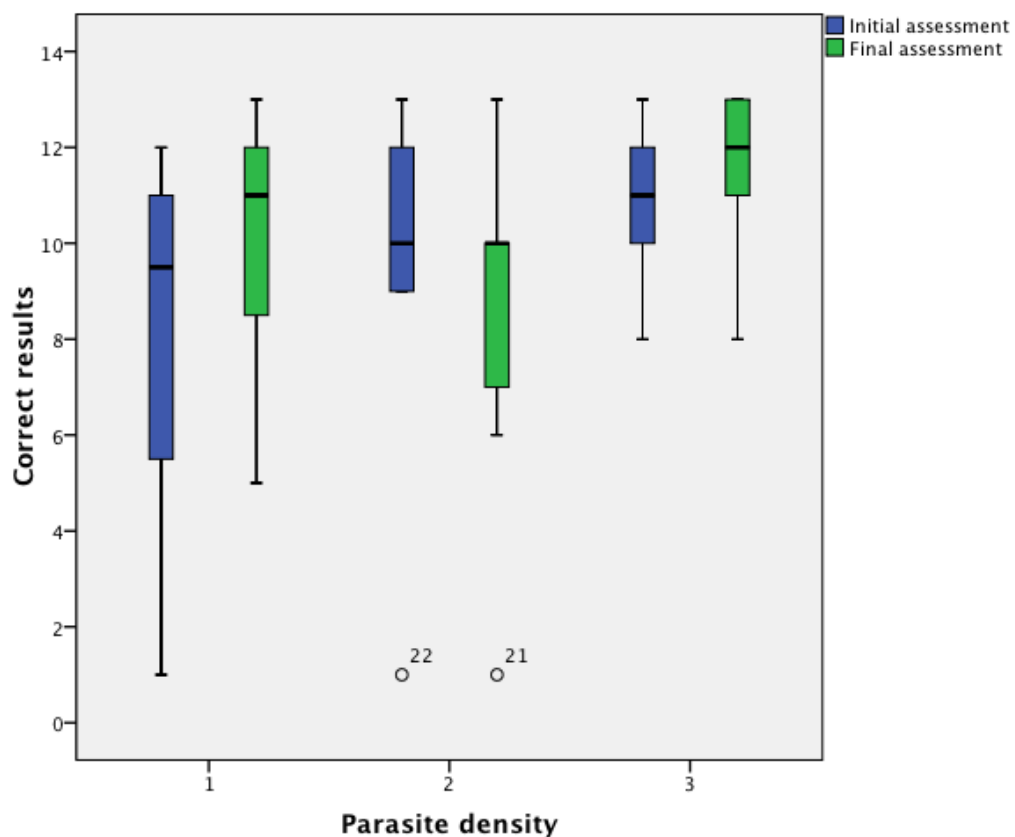
Circles indicate outliers, stars indicate extreme outliers

The species identification accuracy for rank 1 in the initial assessment was 57.1% (± 30.7) and in the final assessment 75.2% (± 20.2). For parasite density rank 2, the species identification accuracy in the initial assessment was 72.0% (± 26.0) and in the final assessment 65.8% (± 29.1). For parasite density rank 3,

the species identification accuracy in the initial assessment was 83.1% (± 11.9) and the final assessment 81.8% (± 24.2).

The species identification accuracy for the different parasite density ranks is shown in figure 5.48. In the initial assessment the parasite density appears to influence the diagnosis made, as the number of parasites increases, the species identification accuracy increases. This trend was lost in the final assessment however, with a worse performance seen on cases with a parasite density of two.

Figure 5.48: Comparison of species identification accuracy and the parasite density in the initial and final assessment



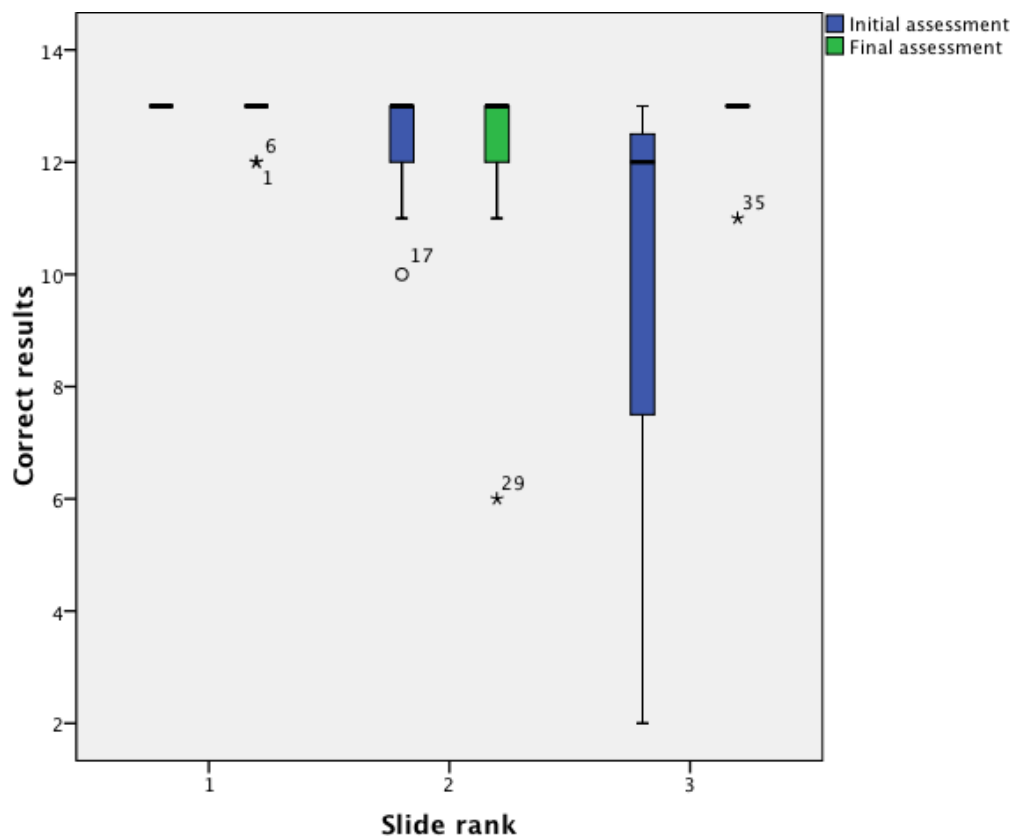
Circles indicate outliers

The median detection accuracy improved slightly for cases at parasite density one and three but fell for parasite density two. The range of these results was the same however.

Overall ranking of the microscopic image

The rank of the microscopic image was also compared to the detection accuracy and species identification accuracy. Figure 5.49 shows the trend in the results, when the detection accuracy of the microscopic image decreases as the rank of the microscopic image increases and the range of the results increases and was deemed to be more difficult. For rank 1, the detection accuracy in the initial assessment was 100% and in the final assessment 98.8% (± 2.9). For rank 2, the detection accuracy in the initial assessment was 93.5% (± 8.0) and in the final assessment 93.9% (± 12.7). For rank 3 the detection accuracy in the initial assessment was 74.7% (± 36.0) and in the final assessment 97.1% (± 5.4).

Figure 5.49: UK group: Comparison of the detection accuracy and the rank of the microscopic image in the initial and final assessment



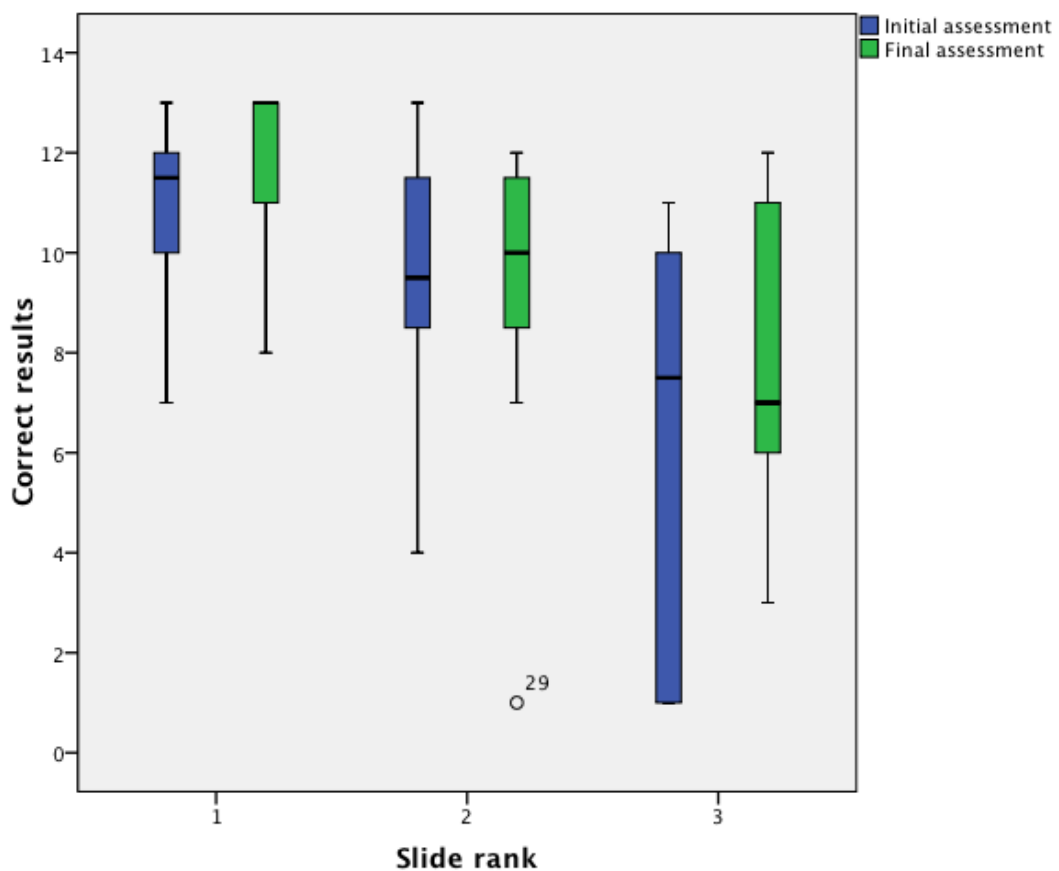
Circles indicate outliers, stars indicate extreme outliers

The species identification accuracy for rank 1 in the initial assessment was 83.3% (± 13.5) and in the final assessment 89.5% (± 15.1). Rank 2 gave a species identification accuracy of 66.7% (± 25.5) in the initial assessment and 74.7% (± 23.5) in the final assessment. Rank 3 gave a species identification

accuracy in the initial assessment of 51.3% (± 36.6) and in the final assessment 54.8% (± 23.1).

Figure 5.50 shows the comparison of the species identification accuracy with the rank of the microscopic image in the initial and final assessment. As in with the detection accuracy, the species identification accuracy decreases as the rank of the microscopic image becomes more difficult.

Figure 5.50: UK group: Comparison of the species identification accuracy and the ranking of the microscopic image in the initial and final assessment



Circle indicates outlier

Presence of artefacts

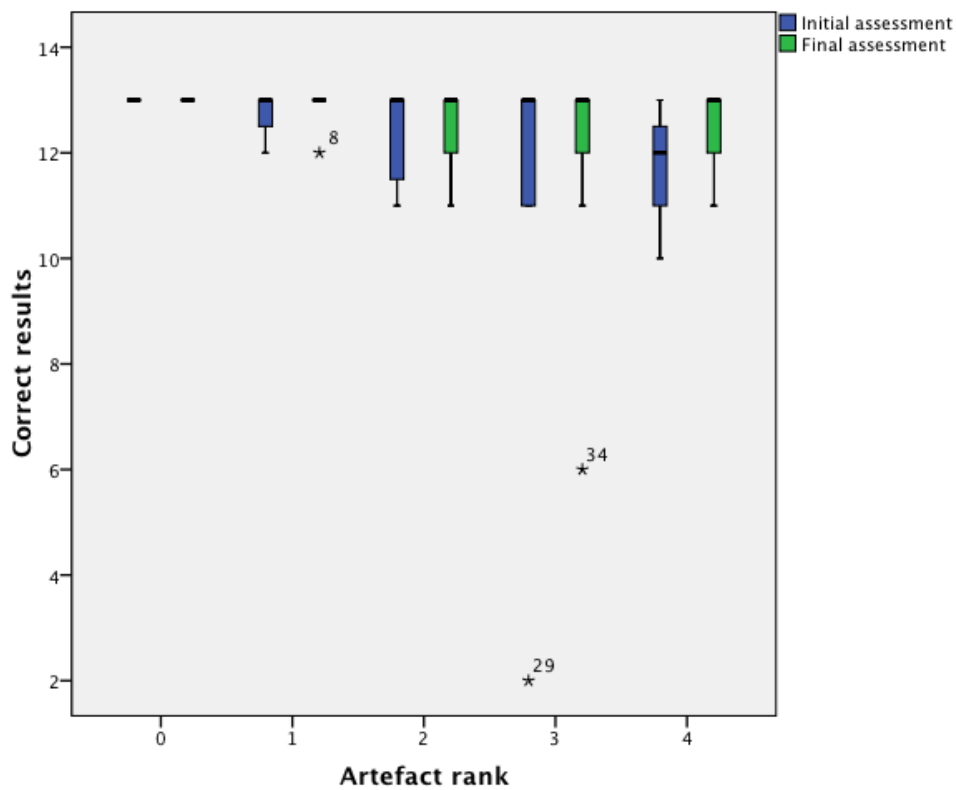
The artefacts on the microscopic image were split into five categories and had relatively small numbers in each group. The artefacts were ranked from zero to four and were compared against the detection accuracy and species identification accuracy in the initial and final assessment. Table 5.27 shows the

detection accuracy and species identification accuracy for the different artefact ranks.

Table 5.27: The detection accuracy and the species identification accuracy of the different artefact rank categories in the initial and final assessment

	Initial assessment		Final assessment	
	Detection accuracy (%)	Species identification accuracy (%)	Detection accuracy (%)	Species identification accuracy (%)
0	98.9 (± 2.9)	72.0 (± 13.5)	100	72.3 (± 27.0)
1	96.2 (± 8.22)	78.0 (± 22.4)	99.0 (± 2.7)	81.7 (± 15.9)
2	94.5 (± 7.3)	71.4 (± 31.0)	96.9 (± 5.4)	80.8 (± 16.0)
3	87.2 (± 27.7)	60.6 (± 29.8)	92.3 (± 14.7)	70.8 (29.0)
4	87.2 (± 22.4)	69.2 (± 35.7)	96.2 (7.7)	65.4 (± 36.9)

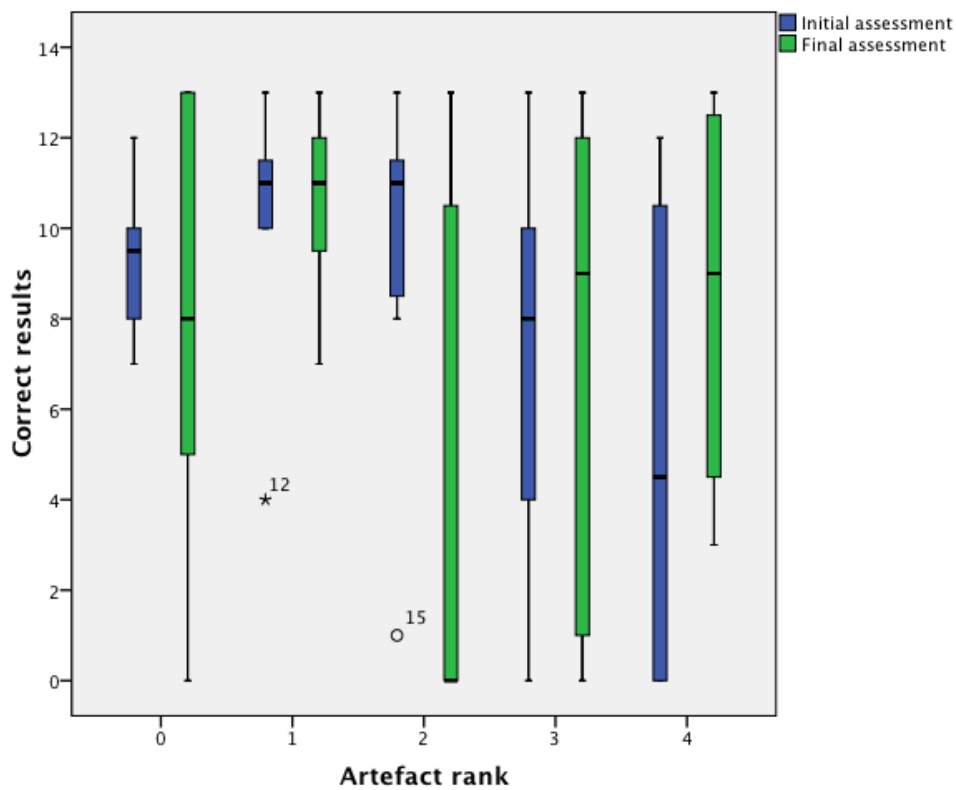
Figure 5.51: UK group: Comparison of the detection accuracy in the presence of artefacts in the initial and final assessment



Stars indicate extreme outliers

Figure 5.51 compares the detection accuracy and the artefact rank in the initial and final assessment. There was little variation in the results in each group and the median was the same in all groups in the final assessment and in all except rank 4 in the initial assessment. There was less variation in the results in the final assessment and less results outside of the normal distribution. The artefact rank therefore had no effect on the detection accuracy of diagnosis for the UK group.

Figure 5.52: UK group: Comparison of the species identification accuracy in the presence of artefacts in the initial and final assessment



Circles indicate outliers, stars indicate extreme outliers

Figure 5.52, gives the comparisons of the species identification accuracy and artefact rank in the initial and final assessment. The median has increased for cases with zero artefacts, but the range of the result has increased between the initial and final assessment.

Comparison of staff undertaking malarial diagnosis by microscopy in the UK group

Table 5.28 shows the comparison of individual results in the initial and final assessment. Out of the 13 participants, there were seven that correctly identified the species in more cases in the final assessment, four with the same number and two with a lower number of correct cases in the final assessment.

The number of incorrect results fell for 11 participants, with one participant staying the same and one increasing from two to three incorrect cases. Four participants had no incorrect results in the final assessment, which was seen as the most important diagnosis, determining whether parasites are present forms the basis of the patient treatment. There were six individuals that increased the number of incorrect species results and six that decreased the number, one participant had the same number throughout.

The most improvement in diagnosing the correct species was seen from participant UK171. The number of correct diagnoses increased from 33 to 38, with no incorrect species in the final assessment, however there were still two incorrect results. The species in the initial assessment were mainly due to the confusion of *P. ovale* and *P. vivax* cases in the initial assessment. UK201 also showed considerable improvement in diagnosis, increasing from achieving the correct diagnosis in 28 cases in the initial assessment to 36 in the final assessment, and reduced the number of incorrect species from nine to four, with no incorrect results in the final assessment.

UK141 increased the number of correctly diagnosed cases from 21 in the initial assessment to 27 in the final assessment. The number of incorrect species fell from 15 cases to ten. The incorrect species determined was different to those in the initial assessment with four of the *P. falciparum* cases being confused with *P. malariae*, only one case was confused in the initial assessment.

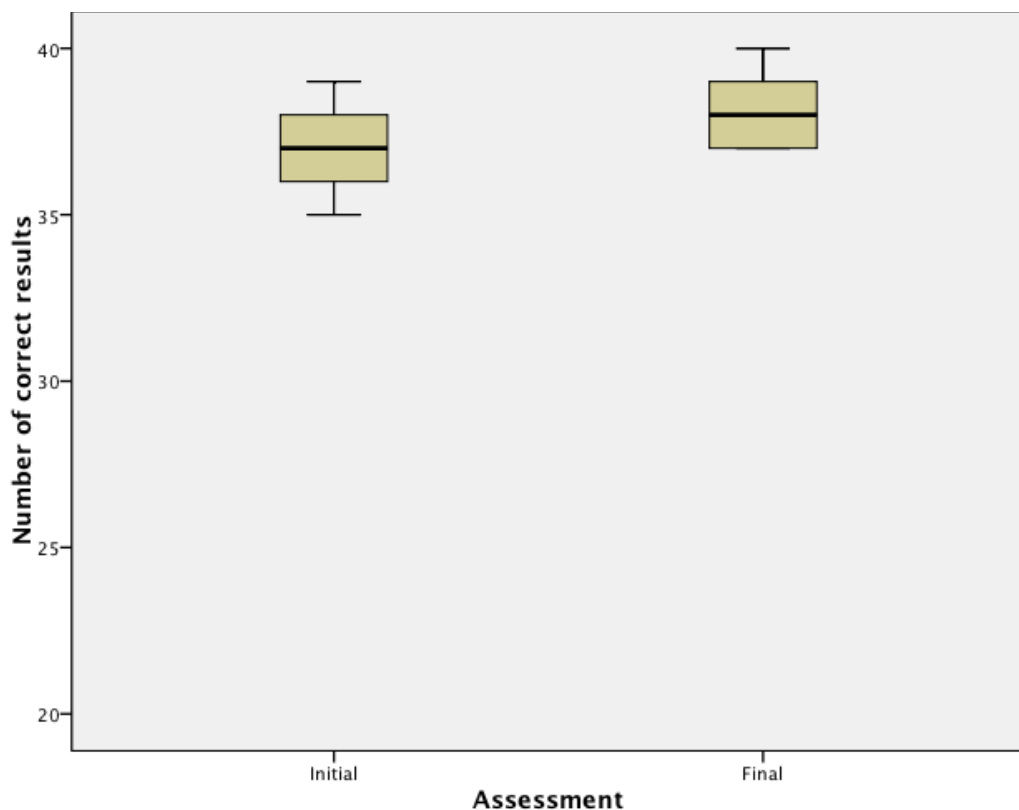
Table 5.28: Comparison of individual participant results in the initial and final assessment

		Initial assessment					Final assessment				
Location	Individual results	Definitive diagnosis			Detection accuracy (%)	Species identification accuracy (%)	Definitive diagnosis			Detection accuracy (%)	Species identification accuracy (%)
		Positive	Negative	Total			Positive	Negative	Total		
1	Positive	123	3	125	92.5	74.2	128	0	128	97.5	81.8
	Negative	9	25	34			4	28	32		
	Total	132	28	160			132	28	160		
2	Positive	31	0	31	95.0	69.7	32	0	32	97.5	66.7
	Negative	2	7	9			1	7	8		
	Total	33	7	40			33	7	40		
3	Positive	62	0	62	95.0	83.3	66	0	66	100.0	95.0
	Negative	4	14	18			0	14	14		
	Total	66	14	80			66	14	80		
5	Positive	62	2	64	92.5	56.1	62	2	64	92.5	68.2
	Negative	4	12	16			4	12	16		
	Total	66	14	80			66	14	80		
8	Positive	120	3	123	90.6	65.9	128	4	132	95.0	68.2
	Negative	12	25	37			4	24	28		
	Total	132	28	160			132	28	160		
Total					93.1	69.9				96.5	76.0

All participants appeared to have more difficulty determining the species on *P. falciparum* cases in the final assessment compared to the initial (figure 5.53).

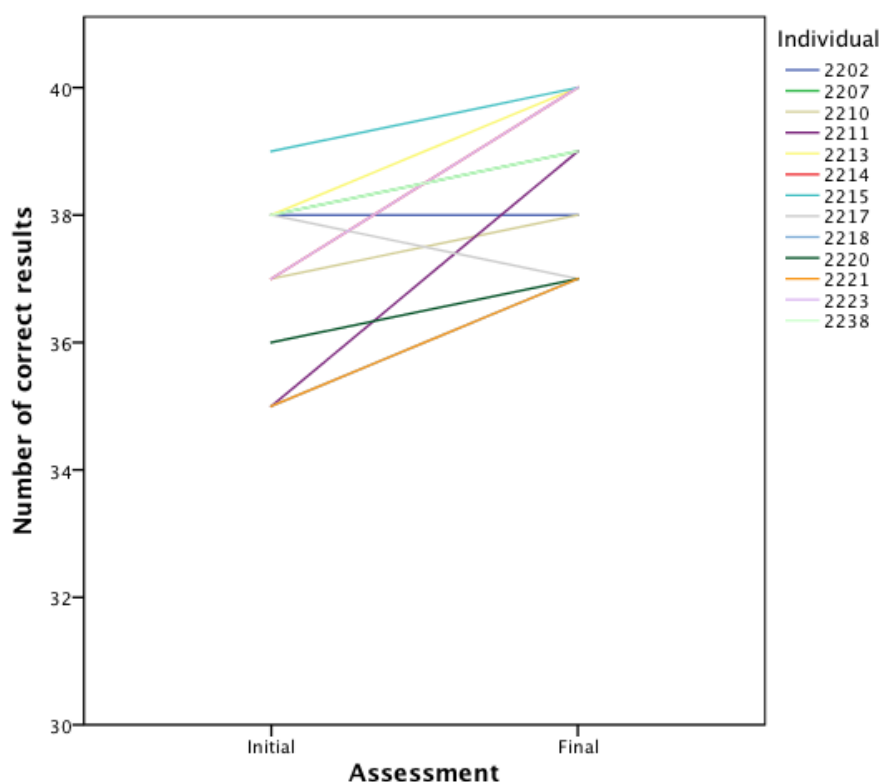
There was a significant difference between the detection accuracy for the individuals ($p=0.005$) in the initial and final assessment (figure 5.53). All participants detected the presence of parasites in more than 37 out of the 40 cases in the final assessment. Four participants detected all parasites present in the final assessment.

Figure 5.53: UK group: Comparison of the detection accuracy in the initial and final assessment



As also described from in table 5.23, figure 5.54 gives the comparison of an individuals performance in the initial and final assessment for determining the presence or absence of parasites.

Figure 5.54: UK group: Individual participant correct results in the initial and final assessment



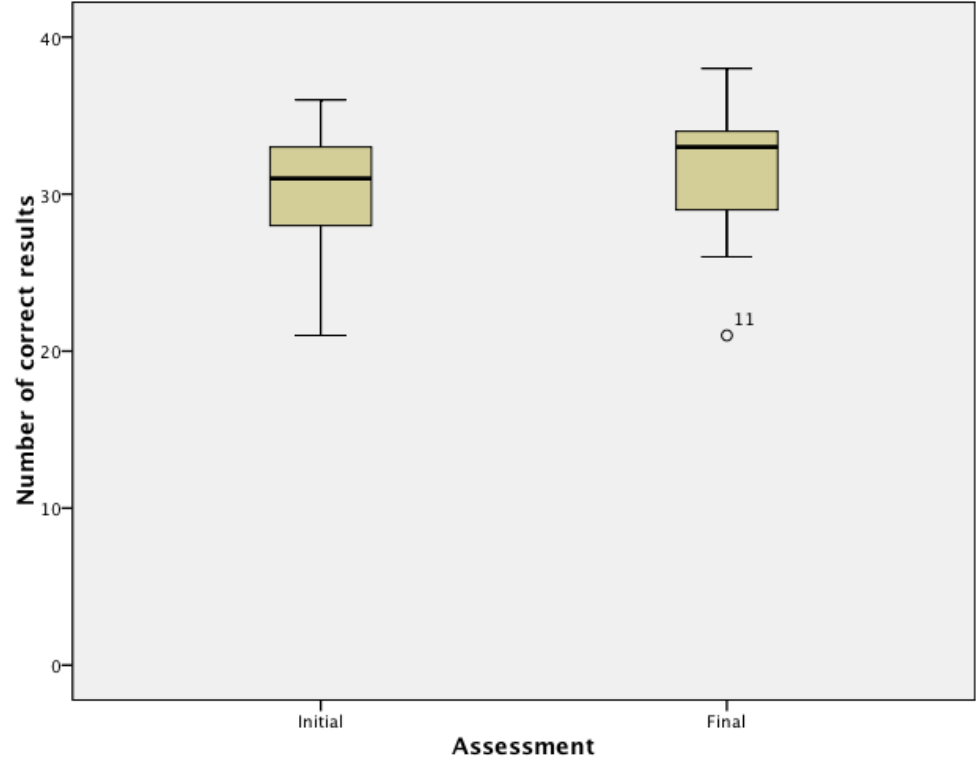
The individuals who have improved their diagnosis can be seen by the increase in the number of correct results. Participant UK161 shows the greatest improvement, with 35 cases correctly identified as positive or negative in the initial assessment and 39 cases in the final assessment.

The comparison of the species identification accuracy in the initial and final assessment is shown in figure 5.55. The median detection accuracy has increased between the initial and final assessment. There was also a smaller range of results in the final assessment.

Figure 5.56 shows the individual performances in the initial and final assessment and shows that while some participants have improved, others have also had difficulty in identifying the species present.

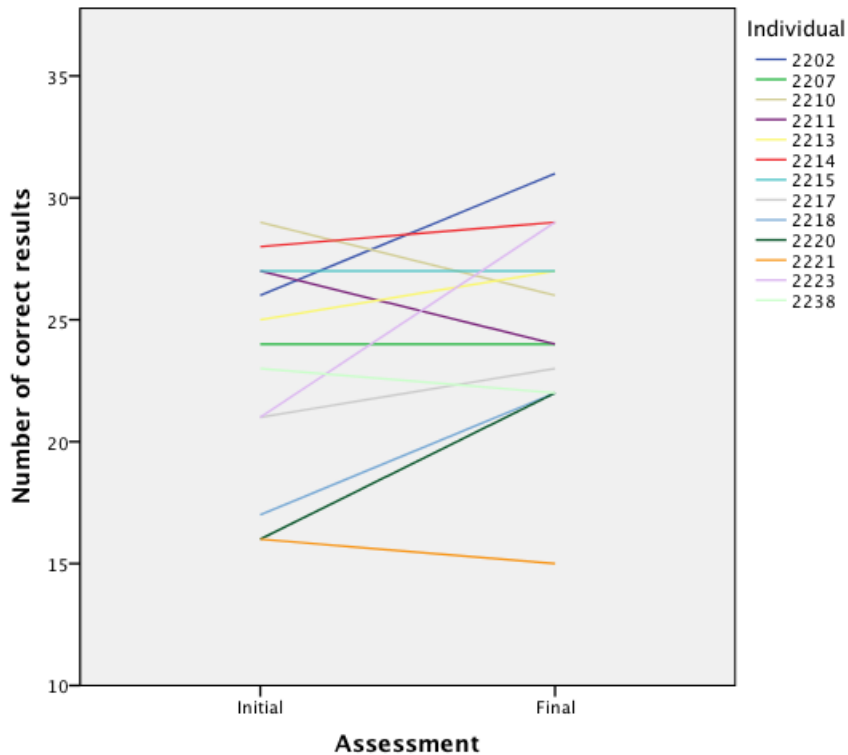
There was a significant difference in the species identification accuracy ($p=0.046$) between the initial and final assessment.

Figure 5.55: UK group: Comparison of the species identification accuracy in the initial and final assessment



Circle indicates outlier

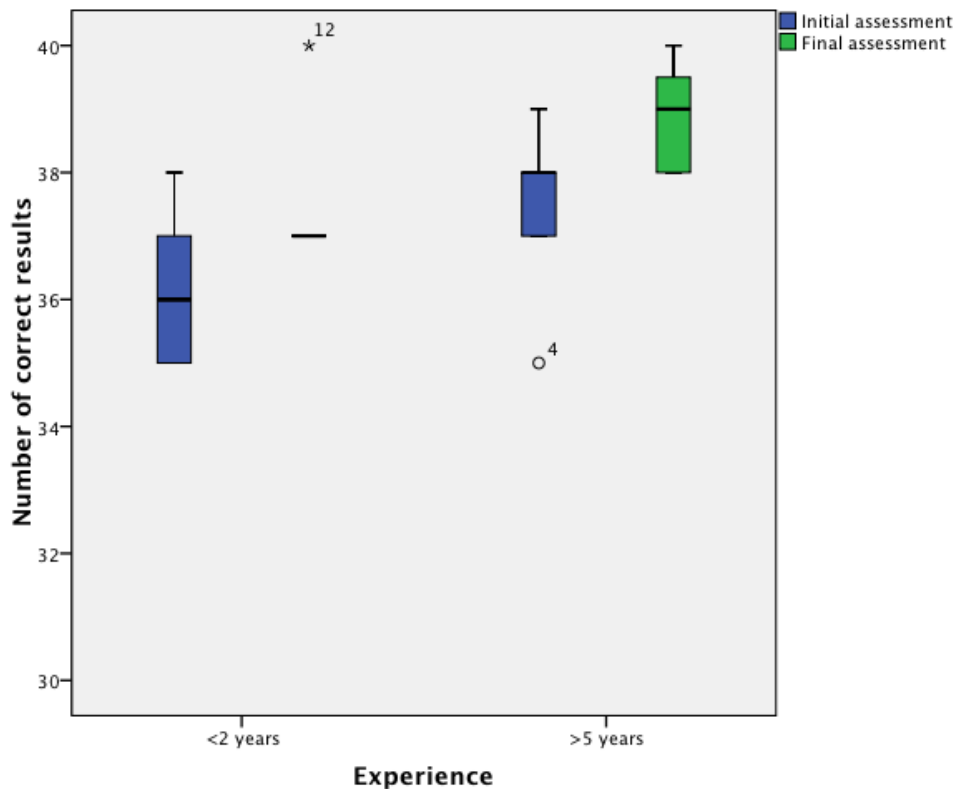
Figure 5.56: UK group: Individual participant correct species results in the initial and final assessment



Experience of the laboratory staff

The individuals were split into two groups of experience, those with less than two years experience and more than five. Figures 5.57 and 5.58 show the comparison of the detection accuracy and species identification accuracy in the initial and final assessment. Figure 5.57 shows an increase in the number of correct results between the initial and final assessment for the individuals in both experience groups.

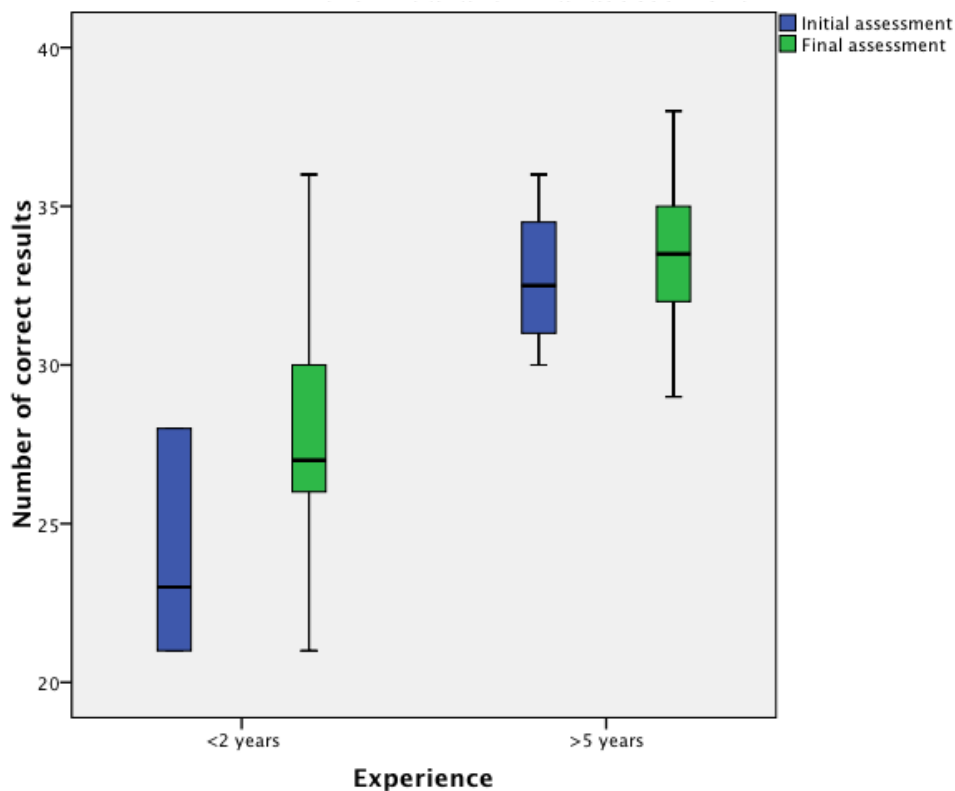
Figure 5.57: UK group: Comparison of the detection accuracy results and the experience of the individual in the initial and final assessment



Circles indicate outliers, stars indicate extreme outliers

Figure 5.58 shows that the species identification accuracy also increased for the participants in the both groups in the final assessment. The improvement was larger for individuals with more than five years experience, indicating that these individuals benefitted more from the training than the more experienced group. The range of results was smaller for the <2 year group in the final assessment than the individual assessment, but was larger for the more experienced group.

Figure 5.58: UK group: Comparison of the species identification accuracy and the experience of the individual in the initial and final assessment



Location of laboratory staff

The location of the individual has no apparent influence of the detection accuracy or species identification accuracy as seen in figures 5.59 and 5.60. Figure 5.59 shows improvement of the individuals, with those at location one and location eight showing the most improvement. There was no improvement in the detection accuracy results at location five, but there was a smaller range of results. Figure 5.60 however shows that the species identification accuracy has improved at location five, but decreased at location two. The species identification accuracy was more variable by location, but this could be due to the experience mix and number of participants at each location.

Figure 5.59: UK group: Comparison of the detection accuracy at different participant locations

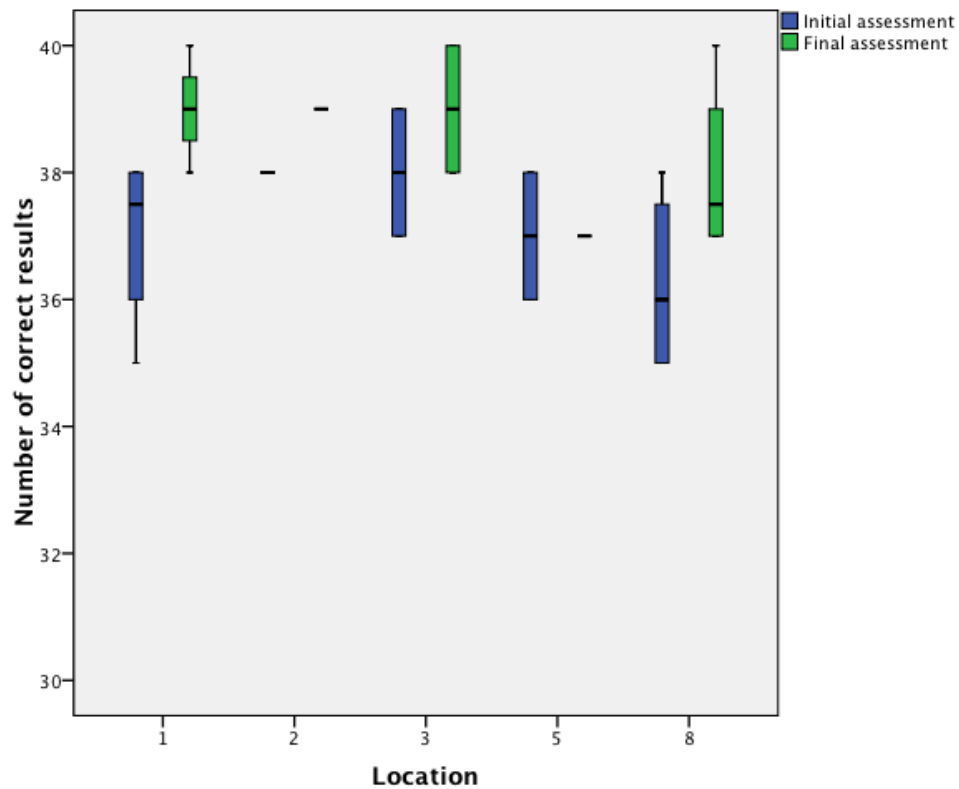
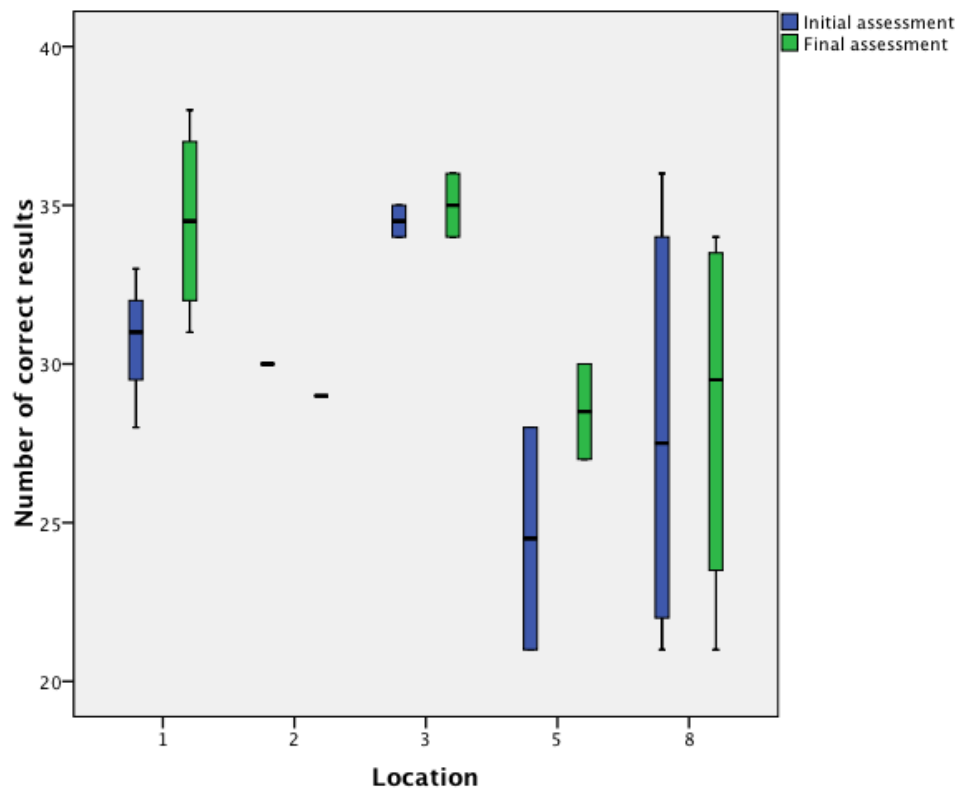


Figure 5.60: UK group: Comparison of the species identification accuracy at different participant locations.



Comparison of participants results on the same microscopic image

Between the initial and final assessment there were five cases that were repeated, however the microscopic image in the final assessment was an inversion of the one used in the initial assessment shown in table 5.29.

Table 5.29: Comparison of results from initial assessment cases that were repeated in the final assessment, for the UK group

Comparison case	Case	Correct results	Incorrect species	Incorrect results
1	30 (initial)	9	4	0
	70 (final)	10	3	0
2	36 (initial)	8	3	2
	72 (final)	6	7	0
3	4 (initial)	11	2	0
	65 (final)	9	4	0
4	33 (initial)	13	0	0
	64 (final)	13	0	0
5	32 (initial)	10	2	1
	79 (final)	12	1	0

The comparison of cases 30 and 70 shows, that one extra participant achieved the correct species on case 70 in the final assessment. Cases 36 and 72 showed fewer participants reached the correct result in case 72 in the final assessment. There were no false negative results in case 72 however.

The consistency of the individuals results between the initial and final assessment was analysed, as shown in table 5.30.

Table 5.30: Comparison of the consistency of results between the five repeated cases for the UK group

Individual	1	2	3	4	5	Total agree
2202	✓	✓	✓	✓	x	4
2207	✓	✓	x	✓	✓	4
2210	✓	✓	✓	✓	✓	5
2211	x	✓	x	✓	x	2
2213	✓	x	✓	✓	✓	4
2214	✓	✓	✓	✓	✓	5
2215	✓	✓	✓	✓	✓	5
2217	✓	x	✓	✓	✓	4
2218	x	x	✓	✓	✓	3
2220	✓	x	✓	✓	✓	4
2221	✓	x	x	✓	x	2
2223	x	x	✓	✓	x	2
2238	x	x	✓	✓	x	2
Total agree	9	6	10	13	8	46/65

Ticks represent agreement. Total number that agree is indicated.

The individuals' agreement between cases 36 and 72 was low, with only six participants agreeing with their own results on the cases. All participants however correctly identified the negative case on both occasions that it was used. Cases 4 and 65 had the highest agreement for a positive case, with ten out of the 13 participants agreeing with their initial diagnosis. Overall, the results are quite consistent, however just inverting an image should not change the interpretation of the image if all of it has been viewed.

Chapter 6: Discussion

To investigate the use of the Internet as a mechanism for the provision of external quality assurance (EQA) and the delivery of training, an intervention study was designed. Virtual microscopy was used to assess diagnostic capabilities pre and post the intervention with an Internet training based training programme.

The overall aim of the project was:

To improve the diagnosis of malaria in the UK and Internationally using the Internet as a training tool and as a provider of EQA, to assess and improve competence

There were a number of objectives:

- To provide high quality digital images for use in quality assessment to take the place of EQA material
- To assess malaria microscopy in the UK and Internationally using the internet as a provider of external quality assurance material via the use of a virtual microscope
- To determine to what extent sample variables such as artefacts and film preparation affect the diagnosis
- To analyse malaria diagnosis at different hospitals within the UK and Internationally to determine if there are any differences
- To assess internet access at the different participating sites, and determine if virtual microscopy is suitable for use in maintaining and improving standards of accuracy in malarial diagnosis.

The results of the overall project demonstrate that for the UK group there were significant differences between the initial and final external quality assessment results and therefore provide proof of the effectiveness of the Internet based external quality assurance and training programme intervention. However, the results of the initial and final external quality assessment for the International group did not prove to be significantly different, indicating that, in this group, the intervention did not improve diagnosis. The reasons for these differences are examined below.

6.1: Production of images for using in training, education and EQA

The methods used for producing the images that were developed throughout the project allowed virtual microscope slides of the highest possible quality to be achieved and provided high quality materials to be used in place of EQA specimens.

High quality photomicrographs of blood cells and parasites were required for both the virtual microscope images and for the image galleries within the training programme. As photomicrographs within the image galleries would be magnified to optimise training, a higher resolution image was necessary. The 12 MPx camera allowed one photomicrograph to be taken for both uses. Once the image was taken, digital sharpening processes were used to achieve the best possible image.

6.1.1: Images used for virtual microscopy

The images used in the initial and final external quality assessments were processed using a detail enhancement protocol and then using contrast masking. These techniques were used to counteract the effect of uploading the image into SlideBox, which slightly degrades the quality.

A number of issues were encountered during image generation, which affected the final quality of the stitched image and the time taken to generate the stitches. Problems encountered included focusing, especially on thick films, as only a single plane image was provided to participants, not all features were focused on every field. This may have influenced the ability to determine whether parasites were present, a future development of the virtual microscope software could be to include z plane focusing, to allow individuals to focus through the plane. Issues with stitching the images were also encountered, Photoshop was used instead of the Axiovision software on the microscope for increased reliability.

Subsequently, modification in the software and an upgrade of the computer has made images easier to capture. The software has been modified to allow each image to be taken at a number of different focus (z) planes, which can then be combined to provide the best focus across the entire image. The stitching method is automated, providing the best overlap to prevent the problems encountered with stitching.

6.1.2: Images used to generate image galleries as part of the training package

To generate the training package a number of small images were required, which would also link to a single microscopic field view. The images would demonstrate features that would usually be examined at x100 magnification (as recommended in World Health Organization, 2009). To achieve this the image collected needed to be at high resolution, preventing pixilation of small features present.

Difficulties were initially faced achieving a good enough image quality for online presentation, because compression of the file was required. The highest possible quality JPEG file was chosen, the images were placed within the pages as links, preventing a loss of image quality when saved within the HTML page.

The 12 MPx images used provided the correct resolution, to enable these high quality images to show small features present, and provided features that are not available in other atlases.

6.2: Use of the Internet to deliver a virtual microscope

There are currently only a few virtual microscope systems capable of delivering large stitched images at high magnification. The only other system in use for parasitology is that of Linder *et al* (2008). As far as the author is aware it was the first time this type of system has been used for image delivery in developing countries, in Africa in particular.

The aim of the project was to create high quality digital images that could be used alongside and replace traditional EQA materials when samples are difficult to obtain and distribute. The images provided in the assessments were deemed to be of a high enough quality to perform this role.

There were problems with the upload of some images, as they would not convert into the SVS format needed to upload them into SlideBox. There appeared to be no reason for this, as some files would work on one occasion and not another.

The size of the image file also became an issue. Using the 12 MPx camera, resulted in the final image being three times as large as it was meant to be

for upload into SlideBox. The software used to convert images into the SVS format used the number of pixels to determine the size of the image.

Therefore, these images had to be reduced to the size of a 1.2 MPx image before upload to be compatible with this system.

The SlideBox system is not available on USB or CD-ROM due to company patents, however an alternative image viewer such as QuickTime could be used to display the images, but it would not have any of the interactive features.

6.3: Production and delivery of the training package

In order to provide a training package, initial research was carried out to determine what was required as part of the training, along with the pedagogical approaches that are required for e-learning. The training was developed to initially provide information, followed by quizzes to provide feedback to the learners. Photomicrographic images were used alongside textual information, for the visual learners. Narratives were used to provide feedback on large stitched virtual microscope slides. The learning was developed throughout the project to improve the interactivity of the training and quizzes. The aim was to design a training programme that was designed on the mastery learning style, however this was not achieved mainly due to a lack of technical knowledge to prevent participants accessing other slides before reaching the desired level of competency. Due to this limitation, the training was modelled on the alternative Miller model of clinical competence (Miller, 1990), using the training to build upon current knowledge and build competence. The training was designed to assess and then build upon knowledge they already had, as they were already diagnosing malaria. Case and image based quizzes were used as part of the training, to cement the knowledge given and feedback was provided to allow the individual to reflect upon their results.

The training package was designed to be delivered both via the Internet and via a local computer on either a USB stick or CD-ROM. To achieve this HTML files were created, which contained all of the text and images for the training. The quizzes were created in Abode Flash, the development of which

was not fully completed before the project had to be delivered to participants and was therefore not as interactive as would have been liked.

The content of the training package is shown in the appendix 1.8.

The review of the training package by 17 external experts, highlighted a number of developments to the training programme, but also emphasised positive and negative points, which could be modified. Sections of the training were rearranged to allow them to provide more in depth information and make it clearer for participants, as well as adding some additional sections. Other suggestions were incorporated into the training, including adding more diagrams and reducing descriptive text. However, there were still further modifications that could have been made to the training if time permitted. As software and the Internet develop, a more interactive approach could have been used, with quizzes presented in a better format, and with the structure of these also being improved. Icons could have been used to navigate the training, rather than textual descriptions, along with a greater variety of photomicrographic images from different blood films to provide a even more realistic range of galleries. The training should also have included discussion forums, to allow those receiving the training to be in conversation with one another.

To allow participants to use the mastery learning approach, a system would have been put in place where the participant could not move onto the next section until the desired level of mastery achieved, i.e. 90-100% on the quiz. The participants could then be allowed to work through the training at their own pace, however due to the limitations of time in the assessment, this may not have been appropriate for the training in the current format.

6.4: The International group

6.4.1: Participant recruitment

Of a total of 42 participants contacted, 37 logged onto the virtual microscope system. Participants in Ghana were lost at this initial stage due to staffing issues and involvement with other projects. Participants in Malawi had an intermittent Internet connection and decided not to take part. In Africa, the Internet is available to only about 5% of the population in Ghana and Malawi

and connection speeds are extremely slow (Miniwatt Marketing Group, 2010). Despite receiving a questionnaire from Chile and Colombia, these participants did not log onto the SlideBox system. The Internet connections in South America are accessible by about 50% of the population (Miniwatt Marketing Group, 2010). Therefore, access may not have been available at all times, the individuals gave email addresses but responded only to contact via the postal system.

Communication with participants in Chile and Colombia was via the postal system, as this was controlled at UK NEQAS to preserve confidentiality, however it caused communication issues. As participants were not contacted directly, they lost some of the interactivity and therefore involvement in the study.

Of the 37 participants that logged onto SlideBox, twenty-five completed all cases in the initial assessment another 12 completed some of the images, including two participants that completed one and three cases respectively. By the beginning of the final assessment 26 participants remained, with 21 of these completing all 40 images. Five participants only completed some of the images, one of these only completing three cases. Eighteen participants completed all the images in the initial and final assessment to allow comparison of the initial and final assessment. Of the participants that were lost, five of these were during the initial assessment and the remaining six participants were lost during the training stage. Four of these participants contacted the author explaining that the Internet access was too slow to continue engagement with the project. Three other participants were in direct contact with UK NEQAS at Watford and were not therefore receiving as much contact as other participants. Participants from India and Kano were lost during the initial assessment. There was no direct contact with participants at Kano, which probably lead to the loss of these participants. Participants at Kano were contacted via a lead individual on the site, but there was confusion over direct communication with the participants as the author did not have the contact details of the individuals and the person on site thought they were being contacted directly. Email addresses at this location were provided to UK NEQAS but were not supplied to the author. As

emails were sent via blind copy, none of the participants knew who was being contacted, which may have prevented information from being passed on.

The number of participants recruited to the project and the number lost throughout weakened the experimental design in that whilst overall numbers remained satisfactory the geographical spread was reduced with the loss of nine countries. The majority of participants recruited were from within Nigeria, with up to six participants at each location. These participants mainly contacted the project team, indicating they were both interested in the project, but also felt that they themselves needed training. The original aim was to involve as many staff as possible from each laboratory, in reality there were only one or two participants from laboratories outside of Nigeria. As participants at other locations were also lost throughout the project, the number of individuals to compare decreased, not allowing the true effectiveness of the training in representative malaria endemic regions to be fully assessed.

A lack of local contacts within Africa and within the five other WHO regions, the Americas, South-East Asia, Europe, Western Pacific and Eastern Mediterranean Region, led to reduced numbers of participants. Some laboratories initially contacted by the investigators demanded monetary payment for their participation and this was deemed unethical by the project team.

Funding Internet access

The provision of funding for Internet access introduced significant delays at the start of the project, taking six months to fully resolve. During the time payments were waiting to be delivered, participants were not able to access the Internet and therefore could not take part in the project, delaying the project for every participant.

Initially banker's drafts were used, generated by the university and sent via the post. There were a large number of delays getting these out of the university, once the forms were submitted it took a further two months until the payments were sent. Once the payments were sent, there were further problems. Many bankers' drafts did not arrive; one participant who received

theirs could not cash it in. Only one participant received and was able to access the money.

Only a few participants had bank accounts, preventing a direct transfer being used. Participants were asked what would be the best mechanism for them; the two most popular routes were Western Union and MoneyGram. Both allowed the transfer of funds, either online, in person at a local shop or via the phone. However, MoneyGram would only transfer to Nigeria via the retail store, which was not applicable in this situation, and therefore Western Union was the only option.

After some consultation within the university, a member of staff authorised to arrange Western Union transfers to take place was found. Another month went by before the participants received any funding. As two payments were to be sent together, another problem was encountered, as Western Union has a limit on the amount that can be transferred within a month. Therefore, the funding provision had to be staged, further delaying the start. The delays and uncertainty that this caused which possibly contributed to the loss of participants during this stage.

6.4.2: Participant engagement

The engagement of these participants depended upon the effectiveness of communication; participants that were in regular contact were more engaged in the project than those who were only in contact infrequently. Some participants also did not evaluate all the images made available to them, meaning that they were not able to be used in the comparison of results, as they had not completed all the cases. There were also two participants that partially completed the images in the initial and final assessment and therefore their results could not be used, as they were incomplete. Some participants reported that they thought they had completed the final assessment, when they had only completed the first 20 images, but did not complete the remaining images as monitored by SlideBox. Communication with participants through UK NEQAS was out of the author's control. Contact could only be made with UK NEQAS who would then pass on the information to the participants. Contact was usually via post, making contact slower and less engaging. Instructions were given via email, but these participants did

not receive all communications and therefore may not have received this information.

To create a timed gateway to the training, access to the files was routed through SlideBox. However participants' individual access history to the training programme could not be monitored in this environment due to anonymity requirements and to avoid bias. SlideBox only allows files to be placed in name order, preventing a structure being developed within the system.

Although the university has a student e-learning environment this is only available to registered students to study modules in standard study programmes, therefore this was not available to be used for this project. At the time of writing, a new system based on Moodle is being implemented in the next 12 months, with a dedicated system for short course learning. These students could have used this system, but the best option available at the time was used. The ideal environment for the training would be a virtual learning environment, where all timelines would be controlled and automatic trigger of emails for deadlines for submission of completed tasks.

Some participants also may have colluded, as individuals from the same site achieved the same results on a majority of cases.

Images were duplicated in the initial and final assessment to check for consistency. The same case was repeated, but was flipped around, moving the parasites to the other end of the image. In some cases moving a small number of parasites when the image was flipped caused difficulties in detection. The difficulty in parasite detection could be due to not examining the entire image. Heat mapping technology could help determine how much of the image being examined.

6.4.3: Results from the International group in the initial and final assessment

In the initial assessment a detection accuracy of 68.9% was achieved and a species identification accuracy of 33.3%. The sensitivity was 64.1% and the specificity 91.3%. In the final assessment, the International group achieved a detection accuracy of 66.3% and a species identification accuracy of 34.7%. The difference in the detection accuracy and species identification accuracy

between the initial and final assessment were not significantly different ($p=0.692$ and $p=0.879$).

The results found in this experiment are in agreement with to others carried out in the same geographical area. In a similar study Ngasala, *et al* (2008) found microscopy to have a sensitivity of 74.5% and a specificity of 59.0%. A positive predictive value of 97.2% and a negative predictive value of 35.1% were achieved by the International participants, with Ngasala reporting values of 53.4% and 78.6% respectively. Ngasala's study was carried out in Tanzania, comparing individuals at the peripheral health centre to those in the reference laboratory. The international group achieved an agreement between individuals of 68.9%. Mitiku *et al*, (2003) achieved an agreement of 75%, similar to that obtained by Ngasala. Reyburn *et al*, (2007) found microscopy to have a sensitivity of 71.3% and a specificity of 92.8%.

The results of this study raise concerns about the quality of malaria diagnosis available, especially with such high numbers of false negative results. The results of this study show that 32% of patients would wrongly be diagnosed and possibly die because of this. Of the *P. falciparum* cases 30% were not detected, this being the most severe malaria could have had serious consequences for the patient. Incorrect species determination could also lead to incorrect treatment, possibly allow resistance to develop but also to the development of hypnozoite forms in *P. ovale* and *P. vivax* causing subsequent reinfection. Results in the final assessment showed little improvement on those in the initial assessment.

6.4.4: Problems provided by case images

The examination of the microscopic images in the initial and final assessment produced a number of problems in parasite detection and identification.

Parasite species

There were differences in the detection accuracy and species identification accuracy on images with different species present. In the initial assessment *P. falciparum* cases had a detection accuracy of 69.4% and a species identification accuracy of 42.4%. These would be the type of cases these participants had most day-to-day experience with and should show their best

performance. However, as the samples had been stored in EDTA, the appearance of the parasites may have been different to what they were used to (Milne *et al.*, 1994). In the final assessment the detection accuracy of *P. falciparum* cases was 71.8% and the species identification accuracy 45.8%. For *P. vivax* cases, a detection accuracy of 85.2% was achieved in the initial assessment, with a species identification accuracy of 22.2%. In the final assessment *P. vivax* cases had a detection accuracy of 35.2% cases and a species identification accuracy of 3.7%. The parasite density of *P. ovale* cases was lower, with participants achieving a detection accuracy of 26.4% and a species identification accuracy of 2.8% in the initial assessment. In the final assessment *P. ovale* cases achieved a detection accuracy of 14.8% and a species identification accuracy of 11.1%. There was only one *P. malariae* case in the initial assessment with few parasites present, the detection accuracy and species identification accuracy achieved were the same at 5.6%. There were two *P. malariae* cases in the final assessment, with a detection accuracy of 13.9% and a species identification accuracy of zero. The detection accuracy on the negative slides was 91.3% in the initial assessment. The detection accuracy on the negative slides in the final assessment was 96.0%.

The species of parasite was only significant for the species identification accuracy (detection accuracy $p=0.227$, species identification accuracy $p=0.010$) in the initial assessment, but was significant for both the detection accuracy ($p=0.022$) and species identification accuracy ($p=0.003$) in the final assessment.

The majority of participants would normally see *P. falciparum* in their routine laboratory work. The performance on these cases was therefore expected to be the best. However, some participants struggled to identify the species present when EDTA storage changes were present. Most participants are used to a freshly prepared sample and may not see EDTA changes in routine practice. Some participants, not recognising these features, called every *P. falciparum* case one of the other species. Some participants may also not have usually carried out speciation, but just determine whether parasites were present, speciation for these individuals therefore caused

some difficulty. An attempt was made to receive samples from the individuals own laboratory, but due to ethical and transport issues, this was not achieved. A previous MSc project (Adewunmi, 2007) showed participants in Nigeria performed better on local slides compared to UK slides.

Difficulty in species identification was notable on the rarer species, which the participants were not familiar with. The most difficulty in speciation was seen on cases with low parasite densities. For example *P. malariae* cases were present at very low parasite density, with only a few parasites present on each image provided. The less parasites present, the less identifying features that are present that allow the species to be easily determined.

Parasite density

In the international group initial assessment, the detection accuracy increased as the parasite density increased. There was a significant difference between the detection accuracy and the ranks of parasite density ($p=0.004$). There was a significant difference between the species identification accuracy and the parasite density ($p=0.012$).

In the final assessment there was a highly significant difference between the detection accuracy and the ranks of parasite density ($p<0.001$). There was a significant difference between the species identification accuracy and the ranks of parasite density ($p=0.025$).

The initial and final assessments demonstrated that the detection accuracy increases as the parasite density increases. In the initial assessment the detection accuracy of diagnosis at the lowest parasite density of <5 cells (rank 1) was 42.1% and in the final assessment 31.6%. For the next parasite density rank 2 (6-49 cells) the detection accuracy in the initial assessment was 64.6% and in the final assessment 56.2%, In the initial assessment for cases with more than 50 parasites present (rank 3) the detection accuracy was 89.4% and in the final assessment 96.5%.

The species identification accuracy for rank 1 in the initial assessment was 23.1% and in the final assessment 22.7%. For parasite density rank 2, the species identification accuracy in the initial assessment was 22.2% and in the final assessment 31.5%. For parasite density rank 3, the species

identification accuracy in the initial assessment was 57.2% and the final assessment 51.5%.

Participants indicated in the recruitment questionnaire that they were used to cases at high parasite density, with the majority of cases having a parasite density of 1-8%. Participants may not have experience of low parasite densities and therefore are more easily missed, which is reflected by the low detection accuracy for rank 1 images. As images were often slow to load on some Internet connections, some participants may not have examined the entire image, with the areas with parasites in possibly not being examined in some cases. The heat mapping technology present in newer versions of the software would have helped in analysing the reasons for this finding allowing determination of what areas of the slide were examined and at what magnification.

Determining the species present on low parasite density cases is more difficult as fewer parasites are present to allow characteristic features to be identified. The most difficulty on low parasite density cases was with species other than *P. falciparum*. Some cases only had a few late trophozoites with characteristic features present, alongside early trophozoites, making diagnosis more challenging.

Thick and thin film preparation

The performances on the thick and thin films were compared. In the initial assessment, the international group showed only a small difference in performance with a better performance on the thin film. On the thin film a detection accuracy of 70.5% was achieved and a species identification accuracy of 33.9%. For the thick film a detection accuracy of 61.1% and a species identification accuracy of 30.0%. The differences between the thick and thin film in the initial assessment were not significant (detection accuracy $p=0.276$) (species identification accuracy $p=0.581$).

The performance on the thick film was worse in the final assessment, but this could have been due to the case distribution as there was a mixed infection case and species present other than *P. falciparum*. The detection accuracy on the thick film was 48.6%, with the species identification accuracy being 18.3%. For the thin film, the detection accuracy was 70.7% and a species

identification accuracy of 39.1%. Speciation is not usually carried out on the thick film however. There were significant differences in the final assessment in the detection accuracy (0.039) and the species identification accuracy ($p=0.021$) between the thick and thin films.

The performance on the thick film may also have been affected by the delivery of the image. The virtual microscope does not give 'z' plane focusing abilities, which is often necessary on the thick film due to the depth of the field. Although parasites were focused upon taking the images, other confusing features could not be focused through to allow the plane of the object to be determined. This could also have been true for some images on from the thin film, but not to as great an extent.

New advances in the microscope technology may improve the quality of the image and prevent parasites being out of focus. The new software upgrade allows the image to be photographed at multiple focus planes, to achieve the best focus over the entire image, as the different planes are merged together.

Artefacts

There were five categories for the presence of artefacts varying by the number of artefacts present. Artefacts included the presence of stain deposit and platelets overlying the erythrocytes. In the initial assessment the ranks of the presence of artefacts caused a significant difference in the detection accuracy ($p=0.026$), however, the species identification accuracy had not reached significance ($p=0.453$),

However, in the final assessment there was no significant difference for the ranks of the presence of artefacts on the detection accuracy ($p=0.606$) and species identification accuracy ($p=0.814$).

The effect of the presence of artefacts appears to have been masked by the parasite density of the cases. There were two particular cases that caused problems in diagnosis, case 28 where parasites were faint and out of focus, and case 29 when *P. falciparum* was present alongside chronic granulocytic leukaemia. In case 29 in particular, participants could have been distracted from the presence of very small parasites at mid parasite density.

Rank of the microscopic image

The cases were ranked for overall difficulty based upon the species, preparation and presence of artefacts, rank 1 being the least challenging and rank 3 the most challenging.

For rank 1, the detection accuracy in the initial assessment was 83.3% and in the final assessment 97.4%. For rank 2, the detection accuracy in the initial assessment was 64.7% and in the final assessment 58.5%. For rank 3, the detection accuracy in the initial assessment was 53.2% and in the final assessment 34.0%.

The species identification accuracy results showed the same trend as the detection accuracy, with the species identification accuracy decreasing as the rank increased and the cases became more challenging. In species identification accuracy for rank 1 in the initial assessment was 50.5% and in the final assessment 54.6%. Rank 2 gave a species identification accuracy of 25.2% in the initial assessment and 30.2% in the final assessment. Rank 3 gave a species identification accuracy in the initial assessment of 18.5% and in the final assessment 15.3%.

In the initial assessment there was a significant difference between both the detection accuracy ($p=0.010$), and the species identification accuracy ($p=0.033$) and the rank of the microscopic image for all categories. In the final assessment there was a highly significant difference between the detection accuracy ($p<0.001$) and the species identification accuracy ($p=0.003$), when compared to the rank of the microscopic image.

The rank of the microscopic image therefore reflects the results seen, with the lower detection and species identification accuracies on the images that are deemed to be the most challenging. The combination of all of the features discussed above make the diagnosis more difficult.

6.4.5: Assessment of performance in relation to the laboratory staff training, experience and laboratory location

The performance in the initial and final assessment and consequently the effectiveness of the training programme was compared to the experience of the individual, time since last received training and the location of the laboratory in which the participants are employed.

Examining the results from some individuals showed improvement, others however showed a decrease in performance. Of the 12 participants that said they completed the training, ten completed all of the initial and final assessment. Two participants showed improvements in the detection accuracy and species identification accuracy results. A further four participants improved their species identification accuracy, but showed a decrease in the detection accuracy.

Training

There were four categories for the time since a participant had last had training, these were all compared using non-parametric statistical analysis. There were 11 individuals in the <1 year group and four in the 1-4 years group. The time that had elapsed since laboratory workers had last received training on the diagnosis of malaria, had a moderate or no effect on the outcome of the diagnosis. In the initial assessment, there was no significant difference in the detection accuracy ($p=0.667$) and the species identification accuracy ($p=0.586$) in comparison to the time training was last received. In final assessment there was no significant difference of the time since last training occurred on the detection accuracy ($p=0.088$) or the species identification accuracy ($p=0.060$).

It can be debated whether the figures for training are accurate, as these were provided by individual questionnaire answers. As this was information provided by the individual and not recorded on a central system, this information is not verifiable.

Experience of the laboratory staff

In the initial assessment, grouping of the laboratory staff by experience demonstrated a positive trend between the detection accuracy and individual experience. However, this did not reach significance ($p=0.104$). There was a significant difference for the species identification accuracy and the experience of the individual ($p=0.009$).

In the final assessment, the results for experience were not significant for the detection accuracy ($p=0.142$) or the species identification accuracy ($p=0.141$).

Geographical location of participants

The locations of the participants were analysed to determine effects on the detection accuracy of diagnosis. Initial analysis by participant location, demonstrates that the laboratories that were involved in external quality assurance (EQA) schemes appeared to have higher detection accuracies and species identification accuracies in the initial assessment. These EQA laboratories were Lebanon, India, Kenya and Hong Kong, with Kano in Nigeria in the process of implementing a training programme.

There was a significant difference in the species identification accuracy in the initial assessment when the participant location was considered ($p=0.006$).

The detection accuracy however had not reached significance when compared with the location ($p=0.094$).

In the final assessment, the results of those from laboratories involved in EQA schemes were better with higher accuracies and species identification accuracies. There was a significant difference in the detection accuracy ($p=0.009$) and the species identification accuracy ($p=0.025$) for the location of the participants. The median results increased but it was difficult to determine whether there was an improvement as different numbers of individuals were involved in the final assessment.

6.4.6: Equipment issues that may have affected performance

Computer screen

The quality of computer screen used can affect the image seen and could affect diagnosis. Participants had difficulty accessing computers and those that they did access were probably of a poor quality. The screens may not have been of a high enough resolution to provide sufficient information to both determine whether parasites are present and then identify the species present. SlidePath advise that the screen resolution is at least 1024x768 pixels (SlidePath Ltd, 2010). This was not recorded, due to participants accessing computers at Internet cafes and therefore may have been different on every occasion.

The screen quality the participants were viewing the images on also could have varied between different sessions. The quality of the image depends on

the viewing equipment as well as the speed of the Internet connection, which makes the diagnosis in this group even more difficult.

Internet connection speed

The speed of the Internet connection could affect the quality of image seen if the image was not fully loaded, the willingness of the participant to examine the image for parasites and identification of the parasite when present.

SlidePath recommend a connection speed of 1 Mbps (SlidePath Ltd, 2010), which many participants would have difficulty reaching as the maximum speeds are only slightly higher, e.g. Nigeria 1.01 Mbps (Ookla, 2010). Many participants reported problems with Internet access, mainly having problems accessing the site, but there were also problems with the loading of the image. Many participants had problems with Flash player, on which the digital microscope is solely based. Throughout the project there were 300 flash problems flagged up by the SlideBox system.

Participants reported that the images for the final assessment took longer to load, however as the images were on the same site, any problems must have been with the local Internet connection used. The participants were also using different computers, as they were reliant on Internet cafes, therefore the connection speed could not be guaranteed.

The speed at which the image loaded could also have affected how much of the image was examined. If an image loaded slowly, it was likely that the participant would only have time to look at a few fields in the stitched image, possibly leading to the high numbers of false negative results on low parasite density samples. The image may also have been examined before it was fully loaded and therefore not in proper focus.

Area of the image examined

The version of the SlidePath software used in this project did not allow tracking of where the individual had examined. It is therefore not possible to determine how much of each image was examined by the participants and therefore why they missed the presence of parasites. Later versions of this software have a heat map facility, which enables monitoring of the participant activity on each slide, at each magnification and how long they spent examining each region. The heat map would give a clearer idea of the

participants' habits when examining an image and give a clearer idea of how false negative results were achieved. The drawback with this system is that it may take longer to load the slide, making the problems faced by participants in this project more profound.

Language difficulties

Another factor that may have influenced the participants' results was their understanding of the task required and of English in general. English was not the first language for any participant, which may have caused difficulties in understanding not only in the information provided, but also in understanding what was required. Without a full understanding of the training structure, it may have been difficult to find the important images and information provided by the galleries. Due to the design of the website, it may also have been difficult to find the training programme, especially if they did not read the associated materials and look at the screenshots provided.

6.4.7: Summary of performance of the International group

The international group showed no improvement in results between the initial and final assessment. The results produced by the participants were similar, although there were a number of categories that were significantly different in the initial assessment and not the final assessment and vice versa. There were significant differences in the parasite density and the rank of the slide for both the detection accuracy and species identification accuracy in the initial and final assessment. The species of parasite was only significant for the species identification accuracy in the initial assessment, but was significant for both the detection accuracy and species identification accuracy in the final assessment. The location was significant for the detection accuracy in the initial assessment, but both the detection accuracy and species identification accuracy were significant in the final assessment. The artefact rank showed a significant difference in the initial assessment for detection accuracy, but was not significant for either in the final assessment. Experience was significant for the species identification accuracy for individuals in the initial assessment, was not significant in the final assessment. The difference between the thick and thin films was not

significant in the initial assessment, but was significant for both the detection accuracy and species identification accuracy in the final assessment.

For this study, the training therefore, does not seem to influence the diagnosis made. Although, the images in the initial and final assessment were chosen to be of the same quality and have the same properties, the international group had more difficulty with these slides.

The result of incorrect diagnosis in clinical practice can result in the death of the patient. Missing *P. falciparum* cases can result in death within a few hours. Incorrectly determining the species can lead to unnecessary treatment (for another condition that malaria was misdiagnosed as or vice versa), along with drug resistance when treatment is provided unnecessarily. A patient with *P. vivax* that is treated for *P. falciparum* will develop hypnozoite liver forms, which can then cause reactivation of parasites and reinfection. The results from these participants are worrying, as many patients would be receiving the wrong treatment.

6.5 The UK group

6.5.1: Participant recruitment

Participants were contacted through UK NEQAS (H) as members that participated in the glass slide scheme for parasite identification. There were 39 participants that were initially recruited, of which 33 completed the recruitment questionnaire. The participants were recruited from ten laboratories around the UK and were split into two groups based on experience. There were 15 participants in the less than 2 years group and 18 in the more than five years group. Twenty-five participants completed all 40 images in the initial assessment. A further seven participants completed more than 30 cases. Eleven participants completed the post-training questionnaire, with thirteen completing all the images in the initial and final assessment. Seventeen individuals completed all the final assessment, but the results of four had to be excluded as they had not completed all of images within the cut off period and therefore had further access to the training.

Due to the reduced timeframe of the project for the UK group, some participants did not have time to complete the assessment stages. The number of participants engaged throughout the project showed similar reductions to the international group, despite the differences in timescale scheduled.

6.5.2: Participant engagement

Participants in the UK group appeared to be more engaged with the project, due to the shortened timeframe compared to the International group.

Participants were in regular contact with the author, asking questions about the different stages of the project.

Some participants asked for further instructions on how to access the training and what was expected of them. Eleven participants completed the questionnaire at the end of the training to say that they had accessed all of the pages in the training.

Some participants were lost throughout the project, mainly due to time constraints. One participant was on holiday throughout the training stage, therefore not being able to complete the final assessment, for this individual the training was made available later, for use as an atlas during diagnosis. This approach was also taken with individuals who had not completed the final assessment. No participant completed this however, possibly as the participants this solution was given to, were already poor responders.

Some participants worked with the help of textbooks, especially in the initial assessment, mainly due to an inadequate knowledge of the parasite species, as they only encountered these in EQA materials.

6.5.3: Results from the UK group in the initial and final assessment

In the initial assessment detection accuracy of 92.3% and a species identification accuracy of 69.9% was achieved. The detection accuracy was generally high, however there were some differences between the different species. In the final assessment detection accuracy of 96.2% and a species identification accuracy of 74.8% was achieved.

There were only a few cases that demonstrated false positive and negative results, however this would still have an effect on the patient. Five per cent of *P. falciparum* cases were still incorrectly diagnosed, more worryingly the

correct species was not achieved in 27% of cases. The incorrect species identification could have therefore led to the incorrect treatment for the patient and possibly the development of drug resistance. This had improved in the final assessment, possibly indicating a need for training amongst laboratory staff. In the UK the effect on the patient may be smaller, as all positive cases are referred for confirmation of diagnosis by PCR, which would see the patient receiving the correct treatment quickly, often a duplicate test is carried out and more than one microscopist examines each case.

There was a significant difference between the detection accuracy ($p=0.005$) and species identification accuracy ($p=0.046$) in the initial and final assessment. The training has therefore been shown to have a significant effect on the diagnosis made, with the number of correctly diagnosed cases improving as well as the number of correctly determined species.

6.5.4: Problems provided by case images

The examination of the microscopic images in the initial and final assessment provided a number of problems in parasite detection and identification for the UK group.

Parasite species

In the initial assessment, the highest detection accuracy was achieved for *P. ovale* cases, with a detection accuracy of 96.2% and a species identification accuracy of 44.2%. In the final assessment, the detection accuracy of the low parasite density *P. ovale* cases was 100%, with a species identification accuracy of 64.1%.

P. falciparum cases had the next highest detection accuracy in the initial assessment, at 94.7% and a species identification accuracy of 73.1%. Milne et al, (1994) reported that 78.6% of *P. falciparum* cases referred to the reference laboratory were correctly diagnosed. In the final assessment, the detection accuracy on *P. falciparum* cases was 98.7%, with a species identification accuracy of 81.7%.

The three *P. vivax* cases in the initial assessment, had a detection accuracy of 71.8% and a species identification accuracy of 43.6%. Milne et al (1994) also reported that 76.6% of *P. vivax* cases were diagnosed correctly. *P. vivax*

cases in the final assessment, had a detection accuracy of 79.5% and a species identification accuracy of 43.6%.

The one *P. malariae* case in the initial assessment, had a detection accuracy of 84.6% and a species identification accuracy of 30.8%. There were two *P. malariae* cases in the final assessment, with a detection accuracy of 92.3% and a species identification accuracy of 80.8%.

The most difficulty in species identification in the UK group was seen between *P. ovale* and *P. vivax* cases. When combining the species identification accuracy for both species in the initial assessment, the species identification accuracy for *P. ovale* increased to 88.5% and for *P. vivax* to 61.5%. Bailey et al (2005) reported that in 2004 on UK NEQAS *P. ovale* case was identified as *P. vivax* by 43% of participants. These species receive the same treatment and therefore the diagnosis made does not influence the patient directly. The same procedure in the final assessment increased *P. ovale* species identification accuracy 98.6% and that of *P. vivax* to 51.3%. Indicating that there was a higher chance that a *P. ovale* case was diagnosed as *P. vivax* rather than vice versa, possibly due to the increased prevalence of *P. vivax*.

Non-parametric statistical analysis was used to determine whether there was a difference in diagnosis seen on cases of the different malarial species, comparing all species. There was a significant difference in the species identification accuracy ($p=0.025$) between the different malaria species in the initial assessment. However, the detection accuracy did not reach significance ($p=0.494$) when compared to the different malaria species. In the final assessment there was a significant difference between both the detection accuracy ($p=0.022$) and species identification accuracy ($p=0.003$) for the different species.

The UK participants see a predominance of *P. falciparum* cases, however as cases are due to international travel, any species can be seen. This could be why the UK group are better at identifying all species and can determine the species on cases that show characteristic features, possibly with the help of textbooks and atlases. Due to the similarity in appearance, confusion

between *P. vivax* and *P. ovale* is commonly seen in EQA schemes (Milne *et al* 1994).

Parasite density

In the initial assessment the detection accuracy increased as the parasite density of the specimens used in the UK group increased. There was a significant difference in the detection accuracy ($p=0.017$) in the initial assessment between the ranks of parasite density. However, the species identification accuracy only approached significance ($p=0.064$).

In the final assessment there was not a significant difference between the values for the detection accuracy ($p=0.196$) or the species identification accuracy ($p=0.071$) and the rank of the parasite density of the case (figure 5.19). The individuals were equally as good at cases of low parasite density than those of high parasite density. Participants are more used to looking for low parasite density cases, as they are often only looking for a single parasite to determine that the patient has malaria.

The initial and final assessments demonstrated that the detection accuracy increases as the parasite density increases. In the initial assessment the detection accuracy of diagnosis at the lowest parasite density of less than five cells (rank 1) was 87.8% and in the final assessment 97.6%. For the next parasite density rank 2 (6-49 cells) the detection accuracy in the initial assessment was 91.6% and in the final assessment 92.3%. In the initial assessment for cases with more than 50 parasites present (rank 3) the detection accuracy was 98.5% and in the final assessment 99.3%.

The species identification accuracy for rank 1 in the initial assessment was 57.1% and in the final assessment 75.2%. For parasite density rank 2, the species identification accuracy in the initial assessment was 72.0% and in the final assessment 65.8%. For parasite density rank 3, the species identification accuracy in the initial assessment was 83.1% and the final assessment 81.8%.

The parasite density of most malaria infections seen in the UK is low, which may explain the small difference between the detection accuracy. The species accuracy is possibly lower on low parasite density cases due to a

lack of parasites present, especially those with characteristic features, which allow the species to be determined easily.

Thick and thin film preparation

The performance on the thick and thin films were compared. The UK group also showed better results on the thin film compared to the thick film. The detection accuracy achieved on the thin film was 96.7%, compared to 71.4% on the thick film. The species identification accuracy on the thin film was 73.1%, with the thick film being 52.3%. There were a few participants who refused to answer the thick film questions and many would not provide a species on the thick film, speciation on the thick film is not common practice in the UK.

In the initial assessment, the detection accuracy was significantly different between the thick and thin films. The species identification accuracy was not significantly different between the thick and thin films.

In the final assessment the differences between the thick and the thin film were also still present. The detection accuracy in the initial assessment on the thin film was 98.6% and on the thick film 86.5%. The species identification accuracy achieved on the thin film was 82.0% and the thick film 48.4%. Bailey et al (2005) reported that the 2004 UK NEQAS results gave the incorrect species in 22.2% of instances, the results here on the thin film exceed this. The results of this study represent a diagnosis made by an individual, however the UK NEQAS results were as a result of a group effort. In the final assessment, both the detection accuracy ($p < 0.001$) and the species identification accuracy ($p = 0.003$) were significantly different.

Thick films are only usually examined in the UK to try to identify parasites present at very low parasite densities. However, some laboratories do not make a thick film, but use a rapid diagnostic kit for this purpose. Participants would not have know how to speciate on the thick film, except for the presence of some characteristic features they would be used to seeing, i.e. crescent shapes gametocytes of *P. falciparum*.

Artefacts

There were five categories for the presence of artefacts varying by the number of artefacts present and the perceived effect these could have on diagnosis.

In the initial assessment, the detection accuracy decreased as more artefacts were present, however there was no trend seen in the species identification accuracy. The results for both the detection accuracy ($p=0.093$) and species identification accuracy ($p=0.382$) were not significantly different when more artefacts were present.

In the final assessment the presence of artefacts appeared to have little effect on the diagnosis made. There was no significant difference in the detection accuracy ($p=0.555$) or the species identification accuracy ($p=0.879$) when compared to the ranks of the presence of artefacts.

There were probably too many categories for the presence of artefacts, giving only small numbers within each group, making it difficult to achieve significance. The UK group, commonly see EDTA changes and artefacts present due to workload management, especially in cases provided by UK NEQAS for EQA purposes, due to the time it takes for these slides to be made and transported to the laboratory.

Rank of the microscopic image

The rank of the microscopic image was also compared to the detection accuracy and species identification accuracy. The detection accuracy decreases and the rank of the microscopic image increases and the case was deemed to be more challenging. The range of the results seen also increases as the rank of the microscopic image increases. For rank 1, the detection accuracy in the initial assessment was 100% and in the final assessment 98.8%. For rank 2, the detection accuracy in the initial assessment was 93.5% and in the final assessment 93.9%. For rank 3 the detection accuracy in the initial assessment was 74.7% and in the final assessment 97.1%.

The species identification accuracy for rank 1 in the initial assessment was 83.3% and in the final assessment 89.5%. Rank 2 gave a species identification accuracy of 66.7% in the initial assessment and 74.7% in the

final assessment. Rank 3 gave a species identification accuracy in the initial assessment of 51.3% and in the final assessment 54.8%.

In the initial assessment the comparison between the three ranks of the microscopic image demonstrated a highly significant difference for the detection accuracy ($p=0.001$), and a significant difference for the species identification accuracy ($p=0.010$). In the final assessment there was a highly significant difference in the species accuracy ($p<0.001$) when compared to the difference between the ranks of the microscopic image. There was a significant difference for the detection accuracy ($p=0.041$) and the rank of the microscopic image.

The UK participants showed the lowest detection and species identification accuracies, on the cases that were deemed to be the most challenging having a rank of three.

6.5.5: Assessment of the performance in relation to the laboratory staff experience and laboratory location

The performance of participants in the UK group in the initial and final assessment, and the effectiveness of the training programme were compared to the experience of the individual and the location of the laboratory in which they work.

Experience

The participants were divided into two groups depending upon their experience. Group one refers to those with less than two years experience or newly registered Biomedical Scientists. Group two is the individuals with more than five years experience, varied from five years up to more than 20 years.

In the initial assessment, the detection accuracy for group one, those with less than two years experience was 90.0% and for group two was 92.1%. The species identification accuracy for group one was 60.5% and group two was 81.9%. This shows that there is a significant difference in the species identification accuracy when compared to the experience of the individual ($p=0.009$). However, the detection accuracy was not significantly different ($p=0.171$) when compared to the individuals' experience.

In the final assessment, the detection accuracy for less than two years experience was 96.6% and for group two was 93.7%. This difference was not significant ($p=0.074$). For species identification accuracy group one was 71.0%, with group two was 75.2%, this difference was however not significant ($p=0.346$).

The training has therefore been shown to have a significant effect on the results of participants in the less experienced group. In the initial assessment there was a significant difference between the results of group one and group two, however in the final assessment there is no longer a significant difference and the results of the two groups are similar. This also indicates that the more experienced group benefitted less from the training.

Laboratory location

In the initial assessment there was no significant difference in detection accuracy results ($p=0.918$) between the different hospitals in which the participants were based. There was no significant difference in the species identification accuracy ($p=0.053$) for the participants location.

In the final assessment there was no significant difference in detection accuracy results ($p=0.618$) between the different hospitals in which the participants were based. The species identification accuracy results were also not significantly different ($p=0.247$) when compared to location.

6.5.6 Equipment issues that may have affected performance

Throughout the project a few issues were encountered with access to the virtual microscope for the UK group. Internet access was sometimes slow and sometimes participants could not access the site.

Internet firewalls

Within the NHS the firewalls sometimes slowed access to the site, and in some cases prevented access to the training. To allow the participants to access the training the software company contacted the IT department at each hospital to ensure access. The firewalls also slowed access, preventing the image being viewed at optimum quality, with some pixilation to the image until it had fully loaded. Problems were also reported with ActiveX control permission, which also for a time prevented access.

Area of the image examined

As for the international participants, UK participants also use the version of the software that did not allow participant activity to be tracked, therefore it was not possible to track how much of the image was examined. The benefits of this inclusion could help this to be determined in the future.

6.5.7: Summary of the performance of the UK group

There was a significant difference between the detection accuracy and species identification accuracy in the initial and final assessment for the UK group. The training therefore appears to have made an improvement in the diagnosis. The improvement seen for the individuals was higher in those with less experience, in the less than two year group than the more than five year group. By the end of the training both groups showed an improvement in diagnosis made, and were achieving similar detection accuracies in the two groups. This could indicate that the participants had all reached the maximum level possible by microscopy or the maximum achievable on these slides. This may also indicate that the more inexperienced staff benefit more from the training than those with more experience.

There were significant differences in the rank of the slide for the detection accuracy and species identification accuracy in the initial and final assessment. The species of parasite had a significant effect on the initial and final assessment for the species identification accuracy, but not the detection accuracy. In the initial assessment, the detection accuracy was significantly different between the thick and thin films. The species identification accuracy was not significantly different between the thick and thin films.

In the final assessment, both the detection accuracy and the species identification accuracy were significantly different for the difference between the thick and thin films. The detection accuracy in the initial assessment showed a significant difference when compared to the ranks of the parasite density, however it was not significantly different for the species identification accuracy. Neither the detection accuracy or the species identification accuracy was significantly different when compared to the parasite density in the final assessment. In both the initial and final assessment for detection accuracy and species identification accuracy the presence of artefact,

experience, location and participation in the training scheme, did not have a significant effect on the results.

The UK group accessed the training over a one month period, and then had access to the images again immediately afterwards. This reduced timescale may allow the benefit of the training to be seen more clearly, but may also indicate that there is a washout period. Participants by the end of the final assessment may have already started to lose some of the information gained. This would require further experimentation to see if it is the case, and indicates that the training could be followed by provision of the material on an atlas basis, using it as a guide whilst making diagnosis. A further group of individuals were recruited for this purpose, but these were already non-returners and they did not engage with the task.

6.6 Comparison of UK and International results

The UK participants achieved a higher detection accuracy and species identification accuracy in the initial and final assessment than the International group. There was a significant difference between the detection accuracy (Initial $p = 0.001$ and Final $p = 0.028$) and species identification accuracy (Initial $p < 0.001$ and Final $p = 0.001$) for the UK and International groups in the initial and final assessments. The UK group showed better performance on the thin film, achieving a detection accuracy of 71.4% in the initial assessment and 86.5% in the final assessment, however their results on the thick film were also better than the International group, with 61.1% in the initial assessment and 48.6% in the final assessment. The numbers of false positive results for these groups were small with 8.3% for the international group and 7.3% for the UK group. However there were less true positive results for the international group with only 64.1% of instances compared to 92.8% for the UK group.

There were also more false negative results in the international group with 35.9% of occurrences, compared to 7.2% for the UK group. There were also difficulties in species determination, the incorrect species was determined in 30.8% instances for the international group and 22.4% for the UK group.

The high number of false negative results in the international group were mainly on low parasite density cases, this could be due to not examining the entire image, possibly linked to a slow Internet connection as discussed earlier.

Although the International and UK groups viewed the same slides and accessed the same training, the timescales of the project were different. The international group accessed the programme over a 14-month period, whereas the UK group accessed over a 4-month period. Due to this time scale, the UK participants may have been more continuously engaged with the project, as there was always something that needed to be done.

Participants communicated, via e-mail, that they were having difficulty finding time to access the training, due to staff holidays, staff absences and workloads. This may indicate that the time periods to access the project were too short. A longer period to access each stage of the training, could have meant that a greater number would have completed the programme. The time of the year of delivery also affects the number of staff in the laboratory, as this training was delivered over the summer vacation period, the number of staff available to participate was reduced. The eventual future training programme would be used as a stand-alone system, and these time limits would not cause difficulties, as limits would be by self-determined participation, not imposed time. It is possible that the international group had too long to access the training, as there were numerous unforeseen delays along the way and deadlines were extended to maintain a viable number of participants. When participants had not completed a stage, the deadline was extended to allow some to complete, but this left others unengaged.

Furthermore, waiting for funding for Internet access caused the longest delays, but the availability of the Internet in some regions also caused problems. The initial timeline proposed was extended a number of times, with participants reporting that they could not access the site. The completion deadlines were also changed, thereby disrupting other participants who had completed on time. Some participants did not complete as they thought they had already completed this stage, possibly due to the changing of deadlines or a lack of understanding in what was required. Most of the activity was in

the last month of the intervention; once participants had been reminded of the limited time they had available to access the training.

The ideal viewing time would depend on the location of the participants and the speed of their Internet connection. A future experiment could be carried out to determine the best timescales for the project, or to be left open for participants to work on in their own time. As the current timescales were deemed to be too long for the International group and too short for the UK group the proposed timescale gives a maximum of ten months. Ten months was the initial timescale planned for the International group, with three months to complete the initial and final assessment and four months for the training. A more robustly structured timetable, with specific deadlines would allow this to be achieved. The participants needed a clearer timescale of what needed to be done in each section, to allow better time management. Possibly because of the timescale given, the UK participants showed a significant improvement in their detection accuracy ($p=0.005$) and species identification accuracy ($p=0.046$) results between the initial and final assessment. There was no improvement seen for the international group, this could have been due to the timescale involved and a possible washout of information. Alternatively, participants may not have fully understood the training because of language difficulties.

It should be taken into account that the UK group may have used textbooks to help them throughout the study, as they are readily available. This may have influenced some of the results seen in the initial assessment and may be why the results from the UK group are better than the International group.

6.7 Comparing participant performance against published performance criteria

6.7.1 Relation to other International studies

The only significant study on the quality of microscopy was carried out by the WHO, in their Malaria Microscopy Quality Assurance Manual (World Health performance of microscopists carrying out routine diagnosis. For the international participants the parasite density calculations were not analysed, due to the large variability in the results received. The WHO recommended

that the assessment was carried out before and after training for a number of different slides, 40 slides were recommended for the ability to detect the presence or absence of malaria parasites and detect the species. The 40 slides included

- 20 negative slides:
 - 20 'clean' negatives
- 20 positive slides of low density (80-200 parasites/ μ L):
 - 10 *Plasmodium falciparum* slides
 - 4 mixed species slides (Include *P. falciparum*. Each species >40 parasites/ μ L, coinfecting species according to local prevalence)
 - 6 of *Plasmodium malariae*, *Plasmodium vivax*, and/or *Plasmodium ovale* slides (include at least 1 of each species, ratio according to local prevalence)
- Time limit: 10 minutes per slide

A further 15 slides were recommended for calculation of parasite density.

These included

- 3-5 *P. falciparum* (200-500 parasites/ μ L,
- 9-10 *P. falciparum* (500-2000)
- 2 *P. falciparum* >100 000 parasites/ μ L)

Time limit: 10 minutes per slide

The grades for accreditation are shown in table 6.1

Table 6.1: Interim WHO grades for accreditation of malaria microscopists

Accreditation Level	Detection of parasitaemia	Species Identification	Parasite Quantitation
Level 1 (expert)	90%	90%	50%
Level 2	80%- <90%	80%- <90%	40%- <50%
Level 3	70%- <80%	70% - <80%	30%- <40%
Level 4	<70%	<70%	<30%

In the initial assessment in this study, only three participants achieved 90% detection of parasitaemia, none of these achieved a species identification of greater than 90%. Therefore, there was one participant who achieved level two, two achieved level 3 and the remainder were in level 4.

The participants were also assessed against the criteria for microscopists at peripheral health centres, which define lower levels of competency (table 6.2).

Table 6.2: Minimum competency levels for peripheral level microscopists as recommended by the WHO

Competency	Result
Parasite detection	90%
Species identification	80%
Accuracy of reporting <i>P. falciparum</i> when present	95%
Quantitation- accurately distinguishing <i>P. falciparum</i> at <10/field and >10/field	80%

Only one participant in the initial assessment achieved these levels of competency, with a species detection of 90% and species identification 80%. There was also little improvement in the results in the final assessment. All participants achieved either a grade of accreditation of three or four and no participant achieved all the criteria for the minimum competency of a peripheral health centre microscopist.

However, there is debate whether the detection of parasitaemia criterion should have a higher threshold. The aim of quality assurance is to ensure that the patient receives the correct diagnosis and treatment. False negative results lead to suffering for the patient; they may not seek further medical advice if their condition deteriorates, leading to increased morbidity and mortality (Amexo *et al.*, 2004), other conditions suffered may include pneumonia and meningitis. To achieve minimal numbers of false positive and false negative results diagnosis needs to be as accurate as possible. Initially competency in sample preparation techniques is required, followed by training in microscopy (World Health Organization, 2009). All microscopists should be able to achieve a high detection rate at high parasite densities and should carefully examine slides at low parasite density. If the minimum requirements are not met, extra training should be provided (World Health Organization, 2009).

6.8: Conclusions and future work

6.8.1: Project conclusions

The Internet has been shown to be suitable as a delivery mechanism for virtual microscopy and for the delivery of EQA specimens. The image produced was of an adequate quality to allow malaria diagnosis to take place. The training programme has also been delivered over the Internet, providing high-resolution images, to allow smaller features to be identified that would be pixelated in lower resolution images.

The training programme has been shown to be effective in improving competence in the UK group, but not in the international group. Due to a lack of detailed participation access data, it is impossible to tell whether this was due to the international group participants having difficulty understanding the content of the training programme or due to a lack of engagement with the programme.

Monitoring participant engagement was a particular problem throughout the project. Access to the SlideBox site was monitored by the investigator, allowing the date and time the participant accessed to be recorded. However how long, or what was accessed, was not. The lack monitoring means it was difficult to determine whether participants had looked at the entire slide or even accessed the training site.

There was no improvement in the results for the international group in the final assessment. The results of participants that did not complete the final assessment were not included in the comparison carried out. In the final assessment the detection accuracy of the international group for the thick film was poorer than in the initial assessment, however the final assessment cases were present at lower parasite density, and there were more cases from species other than *P. falciparum* than in the initial assessment. The main difficulty seen in the international group was on cases of low parasite density, possibly due to the cases they see on a daily basis being mainly of high parasite density. Difficulty was also noted on species other than *P. falciparum* especially in determining the species present.

The UK group showed a significant improvement in results between the initial and final assessment. Those who had less experience in the diagnosis of

malaria showed the most improvement. Improvements were seen in parasite detection on the thick and thin film in the final assessment, however improvements in parasite speciation were only seen on the thin film. The most difficulty in species determination was seen between *P. ovale* and *P. vivax* cases.

6.8.2: Future work

The use of the Internet as a delivery of the virtual microscope has been proven, along with the training package, however as participants still had difficulties in accessing the materials in some locations, an alternative delivery mechanism could be sought. Due to the variability in Internet access between different locations, a CD-ROM or DVD could be used to allow delivery in any location. The mechanism could be trialled with the individuals who did not have an adequate Internet connection to take part.

Delivery of the training programme could also be carried out via mobile phone technology. On a visit to Tanzania, the prevalence of mobile phones that have Internet connections was noted, with connections even in the most rural areas. Most mobile phones now have coloured screens, with smart phones also becoming available. This could be a mechanism of providing the training programme.

The WHO has developed their training manuals (World Health Organization, 2010b) to give both background information and atlas-based material. The training can be provided in groups with the trainer guided by the part 2 manual, or just by following the part 1 manual. The training programme developed during this PhD study was designed to extend on this, providing a gallery of multiple examples, rather than the individual examples of each species and stage provided by the WHO. Using this atlas alongside the microscope can help in the identification of the parasite present. The future use of the training programme could see it used solely as an atlas, with the multiple image examples being compared to the feature of interest seen down the microscope.

As the training programme was not proven to be effective in the international locations used in this study, the training programme could be distributed in the same way to other countries, with an altered format. The format would

aim to achieve mastery, having defined competency targets before allowing participants to progress to the next stage. To provide more extensive competency assessment the virtual microscope would be used to assess whether they can accurately identify the presence of parasites on a larger scale image. A number of examples could be given, if competency was not met on the initial image, a number of others would be provided until this was reached. Feedback would be provided on each image to enhance the training provided, links to specific stages in the training would also be provided to provide the further information required to reach the desired level of competency. Basic competency would initially be assessed in the same way as the initial assessment and the final assessment, to determine whether the training had any influence on the competency. There would not need to be timescales with this setup, as participants would only be able to access the next stage when they completed the previous one.

As there were possible issues with the understanding of the training provided to the international group, the training programme would be translated into the local language of the region in which it is delivered.

Using the new version of the SlideBox software the participant activity could be tracked, allowing more specific information about smear examination and giving more insight about why parasites were missed, or why the wrong diagnosis was made. The feedback could be personalised and could be used as a formative activity to help participants learn where they can improve in the future. Alongside this, a virtual learning environment could be used to engage distant tutors with participants on a one to one basis and to provide them with guidance throughout the project.

The virtual microscope has many other uses, besides the diagnosis of malaria. The virtual microscope could be used for other haematological disorders, parasitology, microbiology and histology learning and competence development. The training programme format could also be expanded to allow other topics or disciplines to be covered.

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Appendix 1.1: USB training programme trial questionnaire

Ease of use and appearance

1. Did you find the information quick and easy to access?

a) Yes ☐ b) No ☐

2. Do the links on the left hand margin allow you to access all the pages you want to?

a) Yes ☐ b) No ☐

If no, what links should be added?

3. Did the pages of the training programme appear correctly on your screen?

a) Yes ☐ b) No ☐

If no, what problems have you encountered?

Concepts and ideas

4. Does the information provided give enough detail to be informative and educational giving enough information to improve malaria diagnosis?

a) Yes ☐ b) No ☐

If no, where do you feel this is lacking?

5. Was the information you expected present?

a) Yes ☐ b) No ☐

If no, what other content did you expect to see?

6. What other content do you feel needs to be included to improve the training given? (Thick film and calculation of parasitaemia information under development)?

7. Is the information provided throughout the USB stick accurate?

a) Yes ☐ b) No ☐

If no, Can you please point out any discrepancies that you feel need to be amended? Please be critical, to allow improvements to be made.

8. Are the images of satisfactory quality to give an accurate representation of parasites on the blood film?

a) Yes ☐ b) No ☐

9. Do the gallery examples of different appearances of the same cell type on the blood film provide an accurate representation of the range of features likely to be seen?

a) Yes ☐ b) No ☐

10. Do you feel the mechanism of delivery is logical?

a) Yes ☐ b) No ☐

11. Would you approach the delivery differently?

a) Yes ☐ b) No ☐

If yes, how would you do this?

12. Any further comments are very welcome

Thank you for your comments and feedback.

Appendix 1.2: Details for case images in the initial and final assessment

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
1	<i>P. falciparum</i>	2	Yes	3	1	Fields thick film, numerous gametocytes present
2	<i>P. ovale</i>	1		2	2	One gametocyte, large platelets surrounding it
3	Negative	-		4	2	Negative slide with many stain deposits which overlay the RBCs and also platelets on RBC
4	<i>P. falciparum</i>	1		3	2	4 late trophozoites, EDTA changes apparent
5	Negative	-		4	2	Negative slide, some stain deposits, some that may be confused with <i>P. falciparum</i> gametocytes. General stain deposit over cells could be confused with stippling
6	<i>P. falciparum</i>	3		0	1	High parasite density, 40/50%, EDTA changes and Maurer's clefts present, some variation in size of infected cells
7	Negative	-		3	1	Negative, with prominent basophilic stippling, possibly leading to the assumption that the stippling is caused by parasites.
8	<i>P. vivax</i>	2		2	2	Early and late trophozoites and two gametocytes. Stippling not prominent, especially in early trophozoites.
9	<i>P. falciparum</i>	3		1	1	High parasite density infection, few artefacts present. Few EDTA changes

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
10	<i>P. vivax</i>	1	Yes	3	3	Few parasites, two prominent late trophozoites, both present at edge of field
11	<i>P. falciparum</i>	2		1	1	Mid parasite density infection, few artefacts, platelets on cells, occasional stain deposit
12	<i>P. falciparum</i>	3		0	1	High parasite density, early and late trophozoites. Some EDTA changes, occasional crenation and Maurer's clefts
13	<i>P. falciparum</i>	3		0	1	High parasite density, many EDTA changes, Maurer's clefts prominent. Accole forms present and large platelets
14	<i>P. falciparum</i>	3		2	1	High parasite density infection, mainly early trophozoites present. Some general stain deposit
15	<i>P. falciparum</i>	2	Yes	2	2	EDTA affected gametocytes on the thick film could be confused with other species, characteristic features also present however.
16	<i>P. falciparum</i>	2		1	1	Mid parasite density, mainly early trophozoites 2 cells with artefacts that could be seen as parasites
17	<i>P. ovale</i>	1		0	1	Low parasite density, all late trophozoites only slightly larger than surrounding cells. Some cells with stippling but no parasites
18	<i>P. falciparum</i>	2		0	1	Mid parasite density, mainly late trophozoites, some EDTA changes pigment present in some late trophozoites

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
19	<i>P. falciparum</i>	3		1	1	High parasite density, malaria pigment in white cells, late trophozoite with Maurer's clefts and many accolé forms. Cells with up to 5 parasites present
20	<i>P. falciparum</i>	3		1	1	High parasite density, late trophozoites present, but no Maurer's clefts evident
21	<i>P. falciparum</i>	1		2	2	Only 2 infected cells, one early trophozoite, one late. Few scratches in slide, some general stain deposit
22	<i>P. vivax</i>	2		0	2	Mid parasite density <i>P. vivax</i> infection. Early and late trophozoite present, stippling prominent, cells are not that enlarged
23	Negative	-		1	2	Negative, few cells with stain deposit/ platelets on top of them
24	<i>P. ovale</i>	1		1	2	Low parasite density, 3 early trophozoites, 2 late. Thick ring in early trophozoite, some stippling. Late lightly stippled cells, lost ring, only slightly enlarged
25	<i>P. falciparum</i>	2		3	2	Mid parasite density, few Maurer's clefts. Some stain deposit all over slide, so one top of cells
26	<i>P. falciparum</i>	2		2	2	Mid parasite density, early and late trophozoites and gametocytes present. Maurer's clefts present in late trophozoites

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
27	Negative	-	Yes	0	2	Negative thick film, lots of platelets present, one stain deposit may be confused with parasite
28	<i>P. falciparum</i>	3		4	2	High parasite density infection, parasites are faint and could be confused with stain deposit. Some are not fully in focus
29	<i>P. falciparum</i>	2		4	2	Mid parasite density infection with very small early trophozoites. Diagnosis is hindered by presence of CGL showing many immature white cells, which distracts from the red cell
30	<i>P. falciparum</i>	2		3	2	Mid parasite density infection, early and late trophozoites present. One cell in prominent Maurer's clefts
31	<i>P. falciparum</i>	1		4	3	Only 2 gametocytes present, are characteristic shape. High platelets and general stain deposit present
32	<i>P. falciparum</i>	1		1	2	One gametocyte present faded, but has characteristic shape
33	Negative	-		4	2	Negative, 2 main stain deposits that may cause confusion, one looking like a trophozoite, the other an accolé form. General stain deposit is present all over slide
34	<i>P. falciparum</i>	2	Yes	4	3	Thick film low parasite density, trophozoites present amongst a lot of background stain deposit

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
35	Negative	-	Yes	4	3	Negative, inadequate lysis, few deposits that could be parasites
36	<i>P. falciparum</i>	2	Yes	3	3	High parasite density thick film, many trophozoites present, some rings thicker. Some red cells fixed so a lot of background deposit
37	<i>P. falciparum</i>	1		4	3	Two parasites present, early trophozoite in a figure of 8 shape and one with two chromatin clefts
38	<i>P. ovale</i>	1		2	2	Two late trophozoites present, some stain deposit on other cells that could be confused with stippling. Infected cells have slight oval shape and are only slightly increased in size
39	<i>P. falciparum</i> and <i>P. ovale</i>	2		3	3	Early and trophozoites, as well as gametocytes for each species. Ovale trophozoites oval shaped, only slightly enlarged.
40	<i>P. malariae</i>	1		3	2	Two gametocytes present, a lot of stain deposit. Gametocytes are faded
41	Negative	-		3	2	Negative film with stain deposit over the slide. Stain deposits are also present on top of the cells, which may be confused with parasites. Some platelet satellitism of the lymphocytes

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
42	<i>P. falciparum</i>	3		1	1	Many small parasites present. Occasional Accole cells but little evidence of Maurer's dots.
43	<i>P. falciparum</i>	1		4	2	Only 3 gametocytes present. One has lost the crescent shape and curled into a ball under the influence of EDTA
44	<i>P. falciparum</i>	2	Yes	4	2	Thick film with background artefacts. Parasites can be seen amongst the background.
45	<i>P. falciparum</i>	3		0	1	High parasite density infection with accole forms and mainly trophozoites present. There are also some cells that have the multiple parasites in.
46	<i>P. ovale</i>	1		1	3	One late trophozoite is present, the rest of the slide has no sign of parasites being present.
47	Negative	-	Yes	2	2	Thick film slide with patches of stain deposit that are too large to be confused with parasites.
48	<i>P. falciparum</i>	3		1	1	High parasite density infection, some parasites are out of focus but are still obviously present. Mainly late trophozoites present, many accole forms present possibly due to EDTA storage
49	<i>P. falciparum</i>	2	Yes	4	3	Thick film, mainly with trophozoites present. Many artefacts present, including some that look like gametocytes.

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
50	<i>P. falciparum</i>	2		2	2	There are only 8 parasites present on this image, all of which are trophozoites. Some platelets are overlaying the red blood cells
51	<i>P. falciparum</i>	3		2	1	High parasite density infection, all trophozoites. Some general deposit over the slide but not to cause confusion in diagnosis.
52	<i>P. falciparum</i>	3		1	1	High parasite density, many cells with more than one parasite in. Some of the trophozoites present have lost their ring shape.
53	Negative	-		2	1	There are some large platelets present on this negative film. A few platelets on overlaying the red blood cells
54	<i>P. falciparum</i>	2		3	1	A combination of trophozoites and gametocytes are present. The gametocytes are on the right of the film, are darkly stained, some of them are distorted from the normal crescent shape.
55	<i>P. falciparum</i>	2		0	1	Small trophozoites present, there are no distinctive characteristics which may cause problems with determining the species.
56	Negative	-		2	1	Negative film, high platelets present. Not many features that would cause problems in diagnosis

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
57	<i>P. falciparum</i>	3		1	1	High parasite density, may accole forms present and EDTA effects as stippling present. There are also some cells with more than one parasite present.
58	<i>P. falciparum</i>	2		3	1	Only gametocytes present, but they have the characteristic crescent shape. Some are slightly distorted, but they the shape is obvious.
59	<i>P. falciparum</i>	3		0	1	Multiple parasites present, but also no staining in the nucleus of the white blood cells. Accole forms are present.
60	<i>P. falciparum</i>	3		0	1	Multiple parasites present, lack of staining in white blood cells. Some platelet clumps. Few cells with Maurer's dots.
61	<i>P. falciparum</i>	1		2	2	Only two parasites present, both of which are gametocytes. Multiple large platelets are present
62	<i>P. falciparum</i>	1		3	2	Only one gametocyte present, some general stain deposit across a lot of the slide.
63	<i>P. malariae</i>	1	Yes	3	2	A thick film with many parasites present, all stages of development are present along with the characteristically shaped daisy ring schizont.

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
64	Negative	-		3	2	Negative, 2 main stain deposits that may cause confusion, one looking like a trophozoite, the other an accolé form. General stain deposit is present all over slide
65	<i>P. falciparum</i>	1		3	2	5 late trophozoites, EDTA changes apparent
66	<i>P. ovale</i>	1		1	2	Five characteristically shaped late trophozoites present as well as pigment within the white cells.
67	<i>P. falciparum</i>	2		1	2	There are six parasites present a combination of early and late trophozoites. There are no obvious artefacts present
68	Negative	-		2	2	Negative film, there are some artefacts present such as large platelets and stain deposit.
69	<i>P. vivax</i>	2		2	2	Numerous parasites present, mainly late trophozoites with stippling present. Parasites are in cells that are only slightly enlarged. There are two gametocytes present, one of which shows all the features clearly.
70	<i>P. falciparum</i>	2		3	2	Mid parasite density infection, early and late trophozoites present. One cell in prominent Maurer's clefts
71	<i>P. vivax</i>	2	Yes	3	2	Thick film with trophozoites present, parasites are small, with unidentifiable features.

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
72	<i>P. falciparum</i>	2	Yes	3	3	High parasite density thick film, many trophozoites present, some rings thicker. Some red cells fixed so a lot of background deposit
73	<i>P. falciparum</i>	1		2	2	Three gametocytes are present, one of which shows EDTA changes, with the curling up of the gametocyte.
74	<i>P. malariae</i>	1		3	2	There are three gametocytes present and one trophozoite. The parasites are small and pale compared to the surrounding cells.
75	<i>P. ovale</i>	1		0	3	There is one gametocyte present at the top of the image. The parasitized cell is larger than the surrounding cells
76	<i>P. falciparum</i>	1		2	3	There are five parasites present, gametocytes show EDTA changes however, with exflagellation. Trophozoites though present are small.
77	<i>P. falciparum</i> and <i>P. ovale</i>	2	Yes	4	3	This thick film shows a mixed infection, gametocytes and trophozoites are present for both species.
78	Negative	-		3	2	Negative film, many artefacts are present including platelets on erythrocytes, stain deposit and other artefacts that resemble parasites
79	<i>P. falciparum</i>	1		1	3	One gametocyte present faded, but has characteristic shape

Case	Case result	Parasite density	Thick film	Artefacts	Rank	Slide summary
80	<i>P. vivax</i>	1	Yes	3	3	Thick film showing two parasites, some white cells also have pigment present. The parasites are difficult to see in the film, with only small parasites present.

Appendix 1.3: International group questionnaire

Laboratory questionnaire

About your laboratory

1. How many people carry out testing in your laboratory?

a) 1 - 2	<input type="text"/>	b) 3 - 4	<input type="text"/>	c) 5 - 6	<input type="text"/>	d) 7 - 8	<input type="text"/>
e) 9 - 10	<input type="text"/>	f) 11 - 12	<input type="text"/>	g) 13 - 14	<input type="text"/>	h) >15	<input type="text"/>

2. How many staff in your laboratory are in each of these categories?

		Number of staff					
		1	2	3	4	5	>5
a)	Laboratory (biomedical) scientists	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
b)	Laboratory technologist	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
c)	Laboratory technicians	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
d)	Laboratory aids	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
e)	Laboratory assistant	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

3. How many staff routinely carry out microscopy to identify blood parasites in your laboratory?

a) 1	<input type="text"/>	b) 2	<input type="text"/>	c) 3	<input type="text"/>
d) 4	<input type="text"/>	e) 5	<input type="text"/>	f) >5	<input type="text"/>

4. Is there a seasonal variation in the number of malaria cases seen?

c) Yes	<input type="text"/>	d) No	<input type="text"/>
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5. If yes, does this variation correspond with a wet and a dry season of the year?

a) Yes ☐ b) No ☐

6. In which months of the year do you see your highest number of cases?
(tick as many boxes as appropriate)

a) January	<input type="checkbox"/>	b) February	<input type="checkbox"/>	c) March	<input type="checkbox"/>
d) April	<input type="checkbox"/>	e) May	<input type="checkbox"/>	f) June	<input type="checkbox"/>
g) July	<input type="checkbox"/>	h) August	<input type="checkbox"/>	i) September	<input type="checkbox"/>
j) October	<input type="checkbox"/>	k) November	<input type="checkbox"/>	l) December	<input type="checkbox"/>

7. How many requests do you have for malaria microscopy in the high season per week?

a) 1 - 9	<input type="checkbox"/>	b) 10 - 29	<input type="checkbox"/>
c) 30 - 49	<input type="checkbox"/>	d) >50	<input type="checkbox"/>

8. How many requests do you have for malaria microscopy in the low season per week (if you have a low season, if not please answer previous question only)?

a) 1 - 9	<input type="checkbox"/>	b) 10 - 29	<input type="checkbox"/>
c) 30 - 49	<input type="checkbox"/>	d) >50	<input type="checkbox"/>

9. What species of malaria do you see most commonly, i.e. in more than 10% of cases?

a) <i>P. falciparum</i>	<input type="checkbox"/>	b) <i>P. vivax</i>	<input type="checkbox"/>
c) <i>P. malariae</i>	<input type="checkbox"/>	d) <i>P. ovale</i>	<input type="checkbox"/>
e) <i>P. knowlesi</i>	<input type="checkbox"/>		

10. What level of parasitaemia do the samples you see most commonly (i.e. in more than 10% of cases) have?

- a) <1% ☐ b) 1-8% ☐ c) >8% ☐

11. What types of tests are carried out in your laboratory? Tick as appropriate

- | | | | |
|--------------------------|--------------------------|-----------------------------|--------------------------|
| a) Malaria
microscopy | <input type="checkbox"/> | b) TB microscopy | <input type="checkbox"/> |
| c) Haemoglobin | <input type="checkbox"/> | d) Blood film
microscopy | <input type="checkbox"/> |
| e) Coagulation tests | <input type="checkbox"/> | f) Full blood counts | <input type="checkbox"/> |

12. Are there any automated haematology analysers within your laboratory?

- a) Yes ☐ b) No ☐

13. If yes, are these analysers fully operational?

- a) Yes ☐ b) No ☐

14. If yes, do you have sufficient access to reagents/ controls to use them on a daily basis?

- a) Yes ☐ b) No ☐

15. If you have any automated analysers, have you been able to get service contracts for them with the manufacturers?

- a) Yes ☐ b) No ☐

About malaria microscopy- microscopes

16. How many microscopes are there in the laboratory?

- | | | | | | |
|------|--------------------------|------|--------------------------|-------|--------------------------|
| a) 1 | <input type="checkbox"/> | b) 2 | <input type="checkbox"/> | c) 3 | <input type="checkbox"/> |
| d) 4 | <input type="checkbox"/> | e) 5 | <input type="checkbox"/> | f) >5 | <input type="checkbox"/> |

17. How many of these microscopes are in a suitable condition for malaria diagnosis?

a) 1	<input type="text"/>	b) 2	<input type="text"/>	c) 3	<input type="text"/>
d) 4	<input type="text"/>	e) 5	<input type="text"/>	f) >5	<input type="text"/>

18. How old are the microscopes being used for malarial diagnosis?

a) <3 years	<input type="text"/>	b) 4 - 10 years	<input type="text"/>
c) >10 years	<input type="text"/>	d) Unknown	<input type="text"/>

19. Are the microscopes serviced regularly?

a) Yes	<input type="text"/>	b) No	<input type="text"/>
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20. Are microscopes monocular or binocular?

a) Monocular	<input type="text"/>	b) Binocular	<input type="text"/>
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21. How many red cells wide is the high power field of view?

a) <30	<input type="text"/>	b) 30-59	<input type="text"/>
c) 60-89	<input type="text"/>	d) >90	<input type="text"/>

About malaria microscopy- staining and slide preparation

22. Which staining methods do you routinely use for malaria parasite staining?

	Giemsa	Fields	Leishman	pH		Other, please specify
				6.8	7.2	
Thick films	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Thin films	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

23. Are slides used for malaria microscopy previously used and then cleaned?

a) Yes	<input type="text"/>	b) No	<input type="text"/>
--------	----------------------	-------	----------------------

24. How is Giemsa stain supplied?

- | | | | |
|------------------|----------------------|------------------------|----------------------|
| a) Powdered form | <input type="text"/> | b) Concentrated liquid | <input type="text"/> |
| c) Dilute liquid | <input type="text"/> | d) Other- please state | <input type="text"/> |

25. If Giemsa stain is made or diluted in the laboratory what type of water is used?

- | | | | |
|-------------------|----------------------|---------------------------|----------------------|
| a) Filtered water | <input type="text"/> | b) Distilled water | <input type="text"/> |
| c) Tap water | <input type="text"/> | d) Double distilled water | <input type="text"/> |

26. If Giemsa stain is used is it filtered before use?

- | | | | |
|--------|----------------------|-------|----------------------|
| a) Yes | <input type="text"/> | b) No | <input type="text"/> |
|--------|----------------------|-------|----------------------|

27. Are thick and thin smears made on the same slide?

- | | | | |
|--------|----------------------|-------|----------------------|
| a) Yes | <input type="text"/> | b) No | <input type="text"/> |
|--------|----------------------|-------|----------------------|

28. How do you ensure staining is carried out at the correct pH?

- | | | | |
|--------------------|----------------------|-------------------|----------------------|
| a) Buffer solution | <input type="text"/> | b) Buffer tablets | <input type="text"/> |
| c) pH meter | <input type="text"/> | d) No control | <input type="text"/> |

29. If methanol is used for fixing the thin film, how long would the slide be placed in methanol for?

- | | | | |
|------------------|----------------------|--------------------|----------------------|
| a) <30 seconds | <input type="text"/> | b) 30 - 59 seconds | <input type="text"/> |
| c) 1 – 2 minutes | <input type="text"/> | d) > 2 minutes | <input type="text"/> |

30. How often is the methanol changed?

- | | | | |
|-----------------|----------------------|-------------------|----------------------|
| a) Not changed | <input type="text"/> | b) Once a week | <input type="text"/> |
| c) Twice a week | <input type="text"/> | d) Every two days | <input type="text"/> |
| e) Every day | <input type="text"/> | f) Twice daily | <input type="text"/> |

31. Does the season or month of the year affect how often you change the methanol?

a) Yes ☐ b) No ☐

32. Are there any controls in place to ensure consistency of slide staining on a day to day basis?

a) Yes ☐ b) No ☐

33. Are any of the staining and slide making processes automated?

a) Yes ☐ b) No ☐

34. Are slides prepared elsewhere before being sent to your laboratory for staining/ testing?

a) Yes ☐ b) No ☐

35. If yes, what proportion of your workload is this?

a) <1%	<input type="checkbox"/>	b) 1 - 4%	<input type="checkbox"/>	c) 5 - 9%	<input type="checkbox"/>
d) 10 - 14%	<input type="checkbox"/>	e) 15 - 19%	<input type="checkbox"/>	f) ≥ 20%	<input type="checkbox"/>

36. If slides are prepared elsewhere, where are they prepared?

a) Other laboratory	<input type="checkbox"/>	b) Health clinic	<input type="checkbox"/>
c) Hospital ward	<input type="checkbox"/>	d) Other, please state	<input type="text"/>

37. If slides are spread or stained outside the laboratory, are they usually satisfactory for the diagnosis of malaria?

a) Yes ☐ b) No ☐

38. How would you rate these slides compared to those spread and stained in the laboratory?

a) Worse ☐ b) Same ☐ c) Better ☐

39. Who prepares slides if prepared outside of the laboratory?

- | | | | |
|-------------------------|----------------------|--------------------------|----------------------|
| a) Health worker | <input type="text"/> | b) Laboratory technician | <input type="text"/> |
| c) Laboratory assistant | <input type="text"/> | d) Other – please state | <input type="text"/> |

40. How long does it take for spread slides to reach the laboratory?

- | | | | |
|---------------------|----------------------|--------------------|----------------------|
| a) <30 minutes | <input type="text"/> | b) 30 – 59 minutes | <input type="text"/> |
| c) 1 hour – 2 hours | <input type="text"/> | d) >2 hours | <input type="text"/> |

41. Who would normally prepare slides for malaria diagnosis in the laboratory?

- | | | | |
|-------------------------|----------------------|--------------------------|----------------------|
| e) Health worker | <input type="text"/> | f) Laboratory technician | <input type="text"/> |
| g) Laboratory assistant | <input type="text"/> | h) Other – please state | <input type="text"/> |

42. Who normally stains slides for malaria diagnosis in the laboratory?

- | | | | |
|-------------------------|----------------------|--------------------------|----------------------|
| a) Health worker | <input type="text"/> | b) Laboratory technician | <input type="text"/> |
| c) Laboratory assistant | <input type="text"/> | d) Other – please state | <input type="text"/> |

43. Are rapid diagnostic tests (kits) for malaria used within the laboratory?

- | | | | |
|--------|----------------------|-------|----------------------|
| a) Yes | <input type="text"/> | b) No | <input type="text"/> |
|--------|----------------------|-------|----------------------|

c) If yes, which kits are used?

44. If used, how are they used, in combination with microscopy or stand-alone?

- | | | | |
|----------------|----------------------|----------|----------------------|
| a) Combination | <input type="text"/> | b) Alone | <input type="text"/> |
|----------------|----------------------|----------|----------------------|

45. How much time would you normally spend looking at blood films to diagnose malaria?

- | | | | | | |
|--------------------|----------------------|--------------------|----------------------|-------------------|----------------------|
| a) <2 minutes | <input type="text"/> | b) 2 – 4 minutes | <input type="text"/> | c) 5 - 10 minutes | <input type="text"/> |
| d) 11 - 14 minutes | <input type="text"/> | e) 15 - 19 minutes | <input type="text"/> | f) >20 minutes | <input type="text"/> |

46. How are samples transported to the laboratory?

- | | | | |
|--------------------|----------------------|-----------------------------|----------------------|
| a) Porter | <input type="text"/> | b) Health worker/ assistant | <input type="text"/> |
| c) Auxiliary staff | <input type="text"/> | d) Driver | <input type="text"/> |
| e) Nursing staff | <input type="text"/> | f) Other- please state | <input type="text"/> |

47. If blood tube samples are accepted, how long after collection would they normally reach the laboratory?

- | | | | |
|---------------------|----------------------|--------------------|----------------------|
| a) <30 minutes | <input type="text"/> | b) 30 – 59 minutes | <input type="text"/> |
| c) 1 hour – 2 hours | <input type="text"/> | d) >2 hours | <input type="text"/> |

48. Are these samples kept refrigerated or at ambient temperature during transport?

- | | | | |
|-----------------|----------------------|------------|----------------------|
| a) Refrigerated | <input type="text"/> | b) Ambient | <input type="text"/> |
|-----------------|----------------------|------------|----------------------|

49. What is the average temperature in the laboratory?

- | | | | |
|----------|----------------------|----------|----------------------|
| a) <25 | <input type="text"/> | b) 25-29 | <input type="text"/> |
| c) 30-35 | <input type="text"/> | d) >35 | <input type="text"/> |

50. Are these samples kept refrigerated or at ambient temperature in the laboratory?

- | | | | |
|-----------------|----------------------|------------|----------------------|
| a) Refrigerated | <input type="text"/> | b) Ambient | <input type="text"/> |
|-----------------|----------------------|------------|----------------------|

51. Do you use any internal quality controls in the examination of malaria films?

a) Yes ☐ b) No ☐

c) If yes what procedures do you use?

52. Do you use any external quality control procedures in malaria microscopy?

a) Yes ☐ b) No ☐

c) If yes what procedures do you use?

About computer and laboratory supplies

53. Is the electricity supply to the laboratory reliable or intermittent?

a) Reliable ☐ b) Intermittent ☐

54. If intermittent how many hours per day do you have a power supply?

☐ Hours

55. Does the hospital have a backup generator?

a) Yes ☐ b) No ☐

56. If yes, how many hours per day does this supply the electricity for?

☐ Hours

57. Do you have times of the day when you have no power supply at all?

a) Yes ☐ b) No ☐

58. Do you have difficulties with obtaining laboratory supplies?

a) Yes ☐ b) No ☐

59. If yes, are these difficulties due to distribution problems?

a) Yes ☐ b) No ☐

60. How many computers are available within your laboratory?

a) 0	<input type="checkbox"/>	b) 1	<input type="checkbox"/>	c) 2	<input type="checkbox"/>
d) 3	<input type="checkbox"/>	e) 4	<input type="checkbox"/>	f) \geq	<input type="checkbox"/>
				5	

61. Is internet access available within the laboratory?

a) Yes ☐ b) No ☐

62. How many of the computers within the laboratory have internet access?

a) 0	<input type="checkbox"/>	b) 1	<input type="checkbox"/>	c) 2	<input type="checkbox"/>
d) 3	<input type="checkbox"/>	e) 4	<input type="checkbox"/>	f) \geq	<input type="checkbox"/>
				5	

63. Does the hospital have an internet subscription?

a) Yes ☐ b) No ☐

64. Is the internet available for you to use?

a) Yes ☐ b) No ☐

65. Is the internet connection lost/ disrupted regularly?

a) Yes ☐ b) No ☐

66. If the internet is lost/ disrupted how long are you able to access the internet per day?

Hours

67. Do you have to use internet cafes to use the internet?

a) Yes ☐ b) No ☐

c) If yes, how much do you have to pay (please state which currency this is in)

Individual questionnaire

About your experience

1. How long have you worked at this laboratory?

a) <1 year

b) 1 - 4 years

c) 5 - 10 years

d) >10 years

2. How long have you been carrying out microscopy for the diagnosis of malaria?

a) <1 year

b) 1 - 4
years

c) 5 - 10
years

d) >10
years

3. Do you have any of the following qualifications relevant to your work?

a) Degree

b) Diploma

--

c) Post-graduate
qualification

--

d) Other relevant
qualification-
state
please

4. What specific training have you been given for malaria microscopy?

a) External training
course

--

b) Demonstration by
another member of
staff

--

c) None

--

d) Other, please state

--

e) If you had specific training, what information and methods were covered?

5. How long ago did you have your last training for malaria microscopy?

a)	< 1 year		b)	1 – 4 years	
c)	5 - 9 years		d)	≥ 10 years	

Calculation of parasitaemia

6. Which method do you use to calculate the level of parasitaemia?

	WBC method	RBC method	Grid method	Other, please state
Thick film				
Thin film				

WBC Method- Used on the thick film. Infected erythrocytes are counted in relation to a number of white blood cells (WBC).

RBC method- Used on thin film. The number of infected cells per 1000 red blood cells (RBC), which is then converted to a percentage.

Grid method- Used on the thick film. A 10 × 10 mm square grid divided into 100 smaller squares etched onto a glass circle fits into the eyepiece of a microscope. In the grid area in 100 high-powered fields is counted, starting counting at the first field. Parasite density is calculated based on volume per field.

7. A total of 156 parasitised cells are counted in 1000 red blood cells, what is the percentage parasitaemia?

Working space

Answer:

Appendix 1.4: UK group questionnaire

1. Please enter your ID code here

2. Do you report blood films?

- ☐ Yes
☐ No

3. How long have you worked in a haematology laboratory for?

- | | |
|------------------------------------|-------------------------------------|
| <input type="radio"/> < 1 year | <input type="radio"/> 11 - 15 years |
| <input type="radio"/> 1 - 2 years | <input type="radio"/> 16 - 20 years |
| <input type="radio"/> 2 - 5 years | <input type="radio"/> >20 years |
| <input type="radio"/> 6 - 10 years | |

4. Have you completed or are you studying for any of the following?

	Completed	Studying for	N/A
Registration portfolio	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Specialist portfolio	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Higher specialist portfolio (MSc)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Advanced higher specialist portfolio	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Diploma of expert practice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

5. What is your staff grade?

- | | |
|--------------------------------------|----------------------------------|
| <input type="radio"/> Trainee BMS | <input type="radio"/> Senior BMS |
| <input type="radio"/> BMS Registered | <input type="radio"/> Chief BMS |
| <input type="radio"/> Specialist BMS | <input type="radio"/> Head BMS |

Other (please specify)

6. What grade of membership do you have with the IBMS?

- ☐ Not a member
☐ Student or associate
☐ Licentiate
☐ Member

☐ Fellow

7. Have you ever attended the UK NEQAS parasitology teaching scheme?

☐ Yes

☐ No

If so please enter date/ year attended

8. On average how many cases of malaria does your laboratory see on a yearly basis?

☐ <5 cases

☐ 6 - 15 cases

☐ 16 - 25 cases

☐ 26 - 35 cases

☐ 36 - 45 cases

☐ >46 cases

Appendix 1.5: Results analysis methods

Participant image evaluation outcome analysis

The following definitions were used for outcome criteria

Correct result- refers to the number of correct answers received, identifying the correct species when malaria parasites present or the absence of parasites when not present.

Partially correct- refers to the number of instances in which parasites are correctly identified as present, but the species is incorrectly identified

Incorrect results- refers to false positive or negative results.

Detection accuracy- the ability of the microscopists to make the correct diagnosis, identifying the presence or absence of parasites, expressed as a percentage.

Species identification accuracy- the ability of the microscopist to identify the correct species of malaria parasite present in the blood film, expressed as a percentage.

Sensitivity- is the proportion of true positives that are correctly identified, expressed as a percentage (ALTMAN and BLAND, 1994b).

$$\text{Sensitivity} = \frac{\text{Number of True positives}}{\text{Number of True positives} + \text{Number of False negatives}}$$

Specificity- is the proportion of true negatives that are correctly identified, expressed as a percentage (ALTMAN and BLAND, 1994a).

$$\text{Specificity} = \frac{\text{Number of True Negatives}}{\text{Number of True Negatives} + \text{Number of False Positives}}$$

Positive predictive value (PPV)- is the proportion of patients with positive test results who are correctly diagnosed, expressed as a percentage (ALTMAN and BLAND, 1994b).

$$\text{PPV} = \frac{\text{Number of True Positives}}{\text{Number of True Positives} + \text{Number of False Positives}}$$

Negative predictive value (NPV)- is the proportion of patients with negative test results who are correctly diagnosed, expressed as a percentage (ALTMAN and BLAND, 1994a).

$$NPV = \frac{\text{Number of True Negatives}}{\text{Number of True Negatives} + \text{Number of False Negatives}}$$

Statistical analysis

Kruskal-Wallis test for independent samples was used for all comparisons in the initial and final assessment using SPSS 18.0.

Comparison results between the initial and final assessment were carried out using Wilcoxon Signed Ranks, again using SPSS 18.0.

Box-plots were also used for comparisons.

Appendix 1.6: Conference poster presentations

Virtual Slides; an exercise in morphology education by UK NEQAS (H) for the World Health Organisation.

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Department of Haematology, Manchester Royal Infirmary (MRI) UK¹, UK National External Quality Assessment Scheme for General Haematology (UK NEQAS (H)) Watford², Manchester Metropolitan University UK³, University of Salford UK⁴, World Health Organisation Geneva Switzerland⁵, School of Tropical Medicine Liverpool UK⁶.

Introduction

UKNEQAS (H) in collaboration with Manchester Royal Infirmary and Manchester Universities have developed a web-based pilot scheme for digital microscopy. Digital morphology is a relatively new tool for promoting education in haematology; we investigated its use in the diagnosis of malaria between different individuals at different laboratory sites within Nigeria. Microscopy is vital for the accurate diagnosis of malaria, and is accepted as the gold standard technique.

Our aim was to investigate the use of large scale high resolution digital images "virtual slides" accessed via the internet. Blood film microscopy for the diagnosis malaria is seen to be highly variable between different laboratories, be it in different countries, or different regions of a country. Malaria diagnosis is highly variable between individuals, mainly depending on the experience and training of the microscopist.

Materials and Methods

Twelve participants from four different laboratories in Nigeria were invited to participate, nine of which completed and returned the associated questionnaire. All laboratories were located in Ibadan, Nigeria, with all participants being laboratory scientists. Participants were based at:

- Malaria clinic, University College Hospital (UCH), (2 participants)
- Haematology department UCH (2 participants)
- Oluoyo Catholic Hospital (2 participants)
- Adeoyo Maternity Hospital (3 participants)




Figure 1: Location of Nigeria and of Ibadan

from UKNEQAS (H), Manchester Royal Infirmary and from Nigerian hospitals, containing a number of variables:

- Positive (8) and negative (5) malaria slides
- Level of parasitaemia (4 low)
- Malarial species (*P. falciparum* (7) and *P. vivax* (1))
- Poor staining (3)
- Thick (6) and thin (7) films
- High platelet counts and other artefacts (3).

All original slides were stained with either Giemsa stain at pH 7.2 or May-Grunwald Giemsa, including those sourced from Nigeria. The process was managed by UKNEQAS (H). The images were distributed over the internet to participants in Nigeria, who completed a questionnaire to assess points such as:

- Whether malaria parasites were deemed to be present and if so what stages of development were present
- What species of malaria parasites were present
- Calculations of parasitaemia and what methods were used to determine these
- General laboratory standards, such as staining methods, and laboratory participation in internal and external quality assurance.
- Internet and computer access within the laboratory and local area.
- The use of rapid diagnostic tests in malaria diagnosis

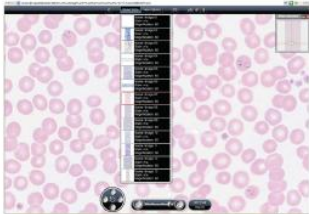


Figure 2: Screenshot from web based image viewer

Results

Of the thirteen cases, 5/8 positive and 1/5 negative were correctly identified by all participants, false positive results were reported in 4 cases by 5 participants, these slides had either high platelet counts or stain deposit, as demonstrated in figure 3.

One participant detected all 8 positive cases, predicted the correct stages in all 8, and determined the correct species in 7/8 cases. Another participant detected all positive cases and negative cases, determining the correct species in 6/8 cases. However, another participant only correctly identified 6/8 positive cases and 4/5 negative cases, determining the correct stage of infection in 5/8 cases and the correct species in 5/8 cases.

4 images had low levels of parasitaemia, 2 of which were correctly identified by all participants. For image 4 (figure 4) 5/9 participants detected the parasites, with another image having 7/9 participants detecting the presence of parasites. The detection rate correlated with the level of parasitaemia.

The species was correctly identified by all participants in 5 cases. Only 4/9 correctly identified one image as *P. falciparum* and only one participant correctly specified the one case of *P. vivax*, image 7 (figure 5).

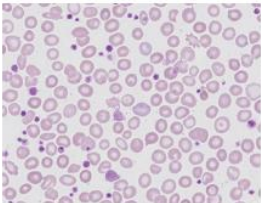


Figure 3: Image 8, a negative sample with a high platelet count and abnormal erythrocytes.

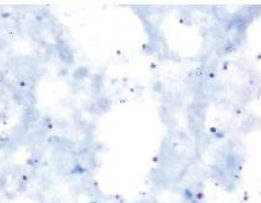


Figure 4: Image 4, *P. falciparum* thick film, with low parasitaemia

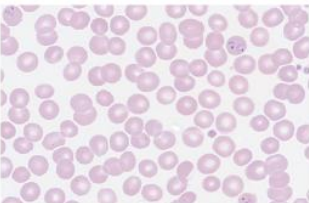


Figure 5: Image 7, *P. vivax* infection, low parasitaemia sample.

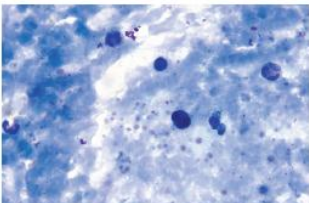


Figure 6: Image 2- poor staining, the thick staining makes the parasites difficult to see.

Summary

Participants in Africa used to digital microscope to identify malaria infection and malarial species in 13 different slides. Assessment of the quality of malarial microscopy has provided scope for future investigations of this nature, as well as to provide information on the possible use of this system as a training and educational tool. Issues with computer access were identified during this study, with only one of the laboratories having direct computer access. However, internet usage in Africa is increasing all the time, as low cost internet connections become available.

This study shows that digital morphology has the potential to be used as an effective training and education tool, reaching professionals beyond the confines of the UK laboratory setting.

Future Direction

Future investigations will be carried out into the use of the system in different countries to assess the quality of malaria microscopy and act as a training and educational tool. Other variables will be investigated including the time required to examine images and pre-analytical variables, such as slide spreading techniques and the re-use slides.

Acknowledgement

All volunteer participants in Nigeria who participated in this pilot investigation.

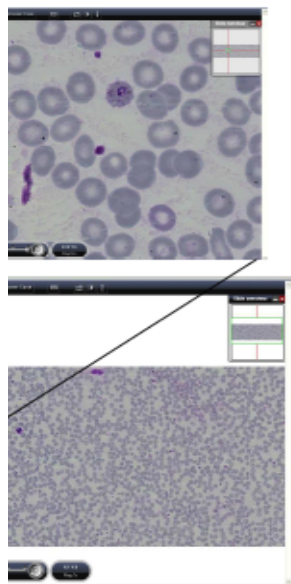
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NB: All images are representative of the procedure; resolution is affected by poster presentation format.

Introduction

ationally recognised gold standard
 inosis; however the ability to reach a
 cted by training, experience and
 ilities, e.g. appropriate stains and
 s.
 ng programme designed to improve
 eing developed, primarily for use in
 pported by the department of Essential
 e World Health Organization.
 ages
 t stage (40 images- 3 months)
 me (6 months)
 it stage (40 images- 3 months)
 sment stage a recruitment
 buted to assess the participants level
 n and availability of internet access.



scope software, showing entire image and

The results of the initial and final assessment stages will be compared to determine whether the training programme has an effect on the diagnosis of malaria.

Materials and methods

The training programme amongst other materials uses "virtual slides," composites of 40 individual images taken using a Zeiss Axio Imager M1 microscope with a 63X oil immersion objective, to replicate the microscope viewing experience.

42 participants have been recruited from 14 laboratories around the world, the majority in Africa.

Participants were recruited using the following criteria

- Malaria cases assessed per week (>10)
- Involvement of a focal person within the laboratory to act as a coordinator
- Willingness to participate in the entire project

English is not the first language for the majority of participants, this has been taken into account throughout the design of the project.

Results

Problems recruiting participants include

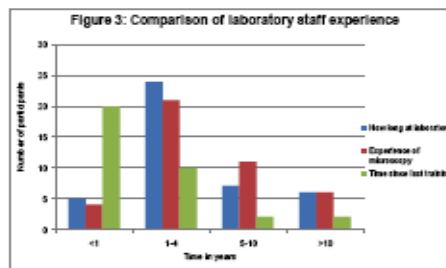
- Continued engagement over a 2 year period
- Expectations of being paid for involvement
- Problems with email communication

Ensuring continued participant engagement throughout the project is a major factor in gaining reliable results for the project.

Internet access responses showed that half of the participants have access to the internet at their place of work.



Figure 2: Locations of laboratories from which participants have been recruited



The remaining participants require funding to use internet cafes, the cost of which varies depending upon the location.

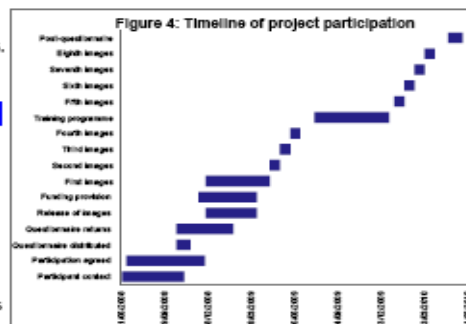


Figure 4: Timeline of project participation

Summary

42 participants have been recruited and a internet based training programme, either hospital, or with the help of funding provic cafes. The training programme being dev improve the diagnosis of malaria, acting r scheme but ultimately as a source of refe diagnosis.

Future direction

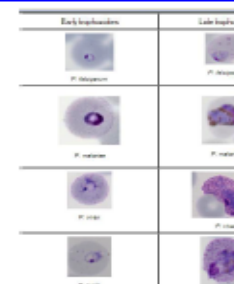


Figure 5: Example of page from the trainin

The assessment stage of the project is no participants having access to 40 images (diagnosis. On completion of this stage th will be made available, consisting of anno alongside an online image atlas and desc quizzes to provide an interactive learning Results of project will be analysed and ca participant experience and training.

Acknowledgements

Thank you to participants for their involve

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Malaria diagnosis assessment and education using virtual microscopy

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Introduction

Microscopy is the internationally recognised gold standard method for malaria diagnosis; however the ability to reach a correct diagnosis is affected by training, experience and adequate laboratory facilities.

An internet based training programme designed to improve diagnostic accuracy is being developed and is supported by the World Health Organization department of Essential Health Technology.

The project has three stages

- Initial assessment stage (40 images- 3 months)
- Training programme (6 months)
- Final assessment stage (40 images- 3 months)

Prior to the initial assessment stage a recruitment questionnaire was distributed to assess the participants level of training and education and availability of Internet access.

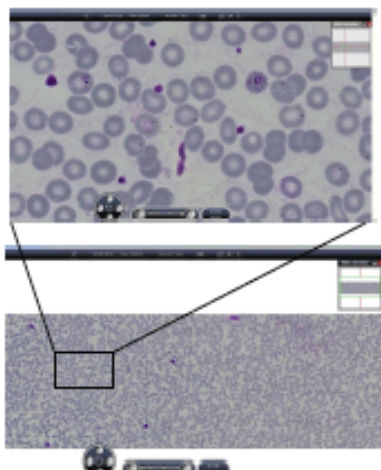


Figure 1: The virtual microscope software, showing entire image and magnified view at 63X

The results of the initial and final assessment stages will be compared to determine whether the training programme has an effect on the diagnosis of malaria.

Materials and methods

The training programme alongside other materials uses "virtual slides," composites of 40 individual images taken using a Zeiss Axio Imager M1 microscope, to replicate the microscope viewing experience. (figure 1)

42 participants have been recruited from 14 laboratories (figure 2), using the following criteria

- Malaria cases assessed per week (>10)
- Involvement of a focal person within the laboratory to act as a coordinator
- Willingness to participate in the entire project

English is not the first language for the majority of participants, this has been taken into account throughout the design of the project



Figure 2: Locations of laboratories from which participants have been recruited

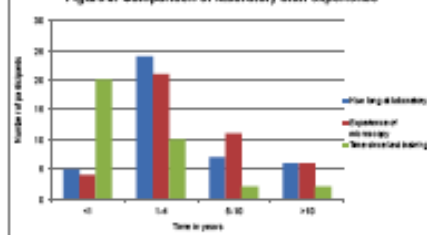
Results

Problems recruiting participants include

- Continued engagement over a 2 year period
- Expectations of being paid for involvement
- Problems with email communication

Ensuring continued participant engagement throughout the project is a major factor in gaining reliable results for the project. Internet access responses showed that half of the participants have access to the Internet at their place of work, with the remainder using Internet cafes.

Figure 3: Comparison of laboratory staff experience

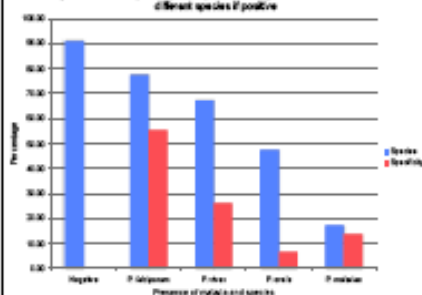


The individuals were asked to assess 40 images for the presence or absence of malaria and to determine the species. 25 participants answered all 40 cases. Others participated to various degrees.

The results varied considerably by slide, depending upon the parasitaemia of the sample, but also on the number of artefacts present. The species present effected the correct speciation, especially in the case of *P. ovale*.

Individuals results also showed variation, with parasite detection ranging from 47.5 to 97.5%. Correct speciation was also difficult with one participant correctly identifying 17.5% of cases.

Figure 4: Sensitivity and specificity of slides negative for malaria and for different species if positive



Summary

42 participants have been recruited and are able to access the internet based training programme, either locally within the hospital, or with the help of funding provided to use internet cafes.

The preliminary results point to a difficulty in speciation, this will now be compared to the participants location to see if this has been influenced by the cases they see on a daily basis.

The individual results will be compared to the results from the questionnaire to see if there is a trend between results and training and experience.

The training programme being developed will aim to improve the diagnosis of malaria, acting not only as a training scheme but ultimately as a source of reference material to aid diagnosis.

Future direction

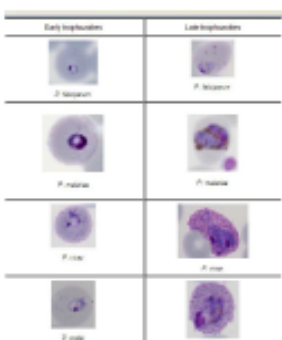


Figure 5: Example of page from the training programme

The training programme is now available to participants, consisting of annotated images alongside an online image atlas and descriptive notes, with quizzes to provide an interactive learning environment.

Results of project will be analysed and categorised against participant experience and training.

Acknowledgement

Thank you to participants for their involvement in this project

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NB: All images are representative of the procedure; resolution is affected by poster presentation format

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PILOT ASSESSMENT OF MALARIA DIAGNOSIS AND EDUCATION USING VIRTUAL MICROSCOPY FOR LABORATORIES IN AFRICA AND DEVELOPING NATIONS

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Introduction

Microscopy is the internationally recognised gold standard method for malaria diagnosis; however the ability to reach a correct diagnosis is affected by training, experience and adequate laboratory facilities.

An internet based training programme designed to improve diagnostic accuracy is being developed and is supported by the World Health Organization.

The project has three stages

- Initial assessment stage (40 Images- 3 months)
- Training programme (6 months)
- Final assessment stage (40 Images- 3 months)

Prior to the initial assessment stage a recruitment questionnaire was distributed to assess the participants level of training and education and availability of internet access.

The results of the initial and final assessment stages will be compared to determine whether the training programme has an effect on the diagnosis of malaria.

Materials and methods

The training programme alongside other materials uses "virtual slides," composites of 40 individual images taken using a Zeiss Axio Imager M1 microscope, to replicate the microscope viewing experience. Annotated images were provided for feedback with links to the training programme (figure 2).

42 participants have been recruited from 14 laboratories in Chile, Colombia, Kenya, India, Hong Kong, Lebanon and Nigeria.

Results

The individuals were asked to assess 40 images for the presence or absence of malaria and to determine the species. 25 participants answered all 40 cases. Others participated to various degrees.

The results varied considerably by slide, depending upon the parasitaemia of the sample, but also on the number of artefacts present. The species present affected the correct speciation, especially in the case of *P. ovale*. Individuals results also showed variation, with parasite detection ranging from 47.5 to 97.5%. Correct speciation was also difficult with one participant correctly identifying only 17.5% of cases.

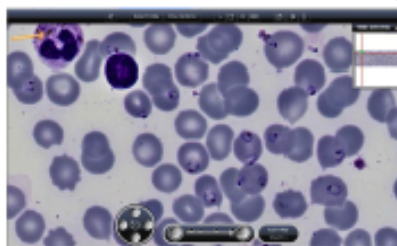


Figure 1: Most accurately diagnosed case, all participants identified malaria was present, all but 2 correctly identified the species

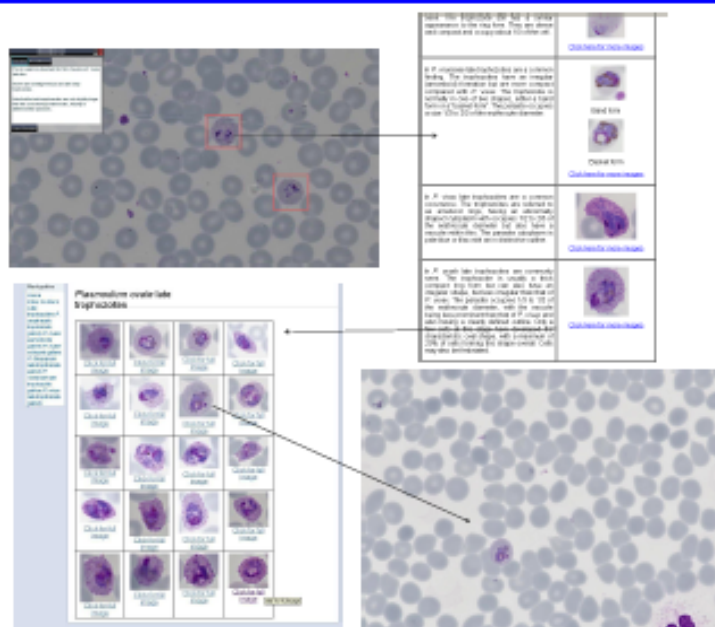
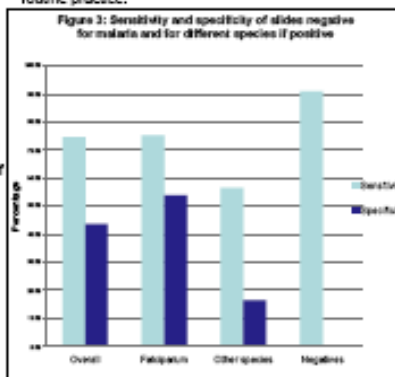


Figure 2: The training programme: Top left an annotated image, this can be linked to training programme, top right. A general page links to the gallery of examples bottom left, which link to a larger image to allow the cell to be seen in context.

Figure 1 shows the case with the most accurate diagnosis, with all participants determining that parasites were present and all but 2 participants gave the correct species. This case is at very high parasitaemia and is closer to what most participants are used to seeing in routine practice.



The training programme provided background information on malaria and information about the appearances of individual species along with single images to emphasise the identifying features as well as giving access the images previously scored in the initial assessment along with annotated feedback. The website gave galleries of examples to show the differences between different cases and species (figure 2). Interactive quizzes with immediate feedback were present throughout.

Participant feedback on the training programme was been positive, with many saying they have improved their

diagnosis. This is currently being assessed in the final assessment stage with images directly comparable to the initial assessment.

Summary

The training programme was been provided to individuals throughout the world. The initial assessment provided highlighted areas in which training was required, especially low parasitaemia cases and species identification.

Future direction

The results from the final assessment stage will be used to determine the effectiveness of the training programme and see if individual's competency has improved. The training is currently being trialed in the UK and with undergraduate students at Manchester Metropolitan University.

Acknowledgement

Thank you to participants for their involvement in this project

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1. Bates, I., Bates, V. & Awan-Adji, A. (2004) Improving the accuracy of malaria-related laboratory tests in Ghana. *Malaria Journal*, 3, 38-43.
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Appendix 1.7: Conference presentation: Sysmex users symposium 2010

Digital Morphology: A training tool for the UK and Developing Nations

Digital morphology has been used in a similar format for training of laboratory staff based in Africa and laboratories involved in the International External Quality Assessment scheme run by UK NEQAS. Participants were based in Kenya, Nigeria, Chile, Colombia, Hong Kong, India and Lebanon.

Participants were initially asked to make a diagnosis on 40 blood smear images to determine the presence or absence of malaria and to identify the species present. These participants were then given access to a training programme over a six-month period, before completing another assessment of 40 images. The images in the initial and final assessment were chosen to match a set of criteria, to allow the effectiveness of the training to be determined.

The training programme, an internet based training package was delivered over six months, providing annotated feedback along with detailed information and images of cells containing parasites from the different species and stages of infection. Quizzes were also used to allow immediate feedback throughout the training.

Forty-two participants were recruited from 14 laboratories recommended by the WHO, UK National External Quality Assessment Scheme for general haematology (UKNEQAS (H)) and the Liverpool School of Tropical Medicine. Of the 42 participants, 24 completed all 40 cases in the initial assessment, another 15 completing various parts. Twenty-one participants completed all 40 cases in the final assessment. The comparison of results in the initial and final assessment were carried out for 18 participants, who completed all the images in the initial and final assessment.

Results from the initial assessment indicate that the correct diagnosis was made in 68.8% of cases, with the correct species being identified in 33.2%.

The final assessment indicated that the number of correct diagnoses made was unchanged at 66.3% and there was also no change in the species determination at 34.7%. Although individual features within the analysis did improve, there was no evidence that the training programme improved diagnosis.

The training programme has been shown to be effective in individuals in the UK, therefore the reasons for not being effective in Developing Nations have to be determined. These could be due to difficulties in understanding English, speed of internet connection, computers being used or the compliance of the participants.

Appendix 1.8 DVD of training programme and images